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FACTORS AFFECTING INFECTION OF WHEAT HEADS BY GIBBERELLA SAUBINETII[1]

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INTRODUCTION

The major portion of the work upon which this report is based was carried on in 1920, 1923, and 1924 as part of a general study of the diseases of cereals caused by *Gibberella saubinetii* (Mont.) Sacc. and by certain species of Fusarium. The present paper describes inoculation experiments and histological studies of wheat scab, or head blight, caused by *G. saubinetii*, together with such macroscopic observations as have a direct bearing on the host-fungus relationship.

WHEAT SCAB A FLORAL INFECTION

According to Palchevsky (*6*),[3] Woronin in 1889 observed that wheat kernels infected with the conidial stage of *Gibberella saubinetii*, which he called *Fusarium roseum* Lk., were considerably lighter in weight than normal kernels. Woronin did not, however, investigate the method of infection or the factors influencing the severity of the disease.

In 1891, Arthur (*2*) reported that wheat scab is a floral infection. He states that the spores of the fungus blow through the air and lodge on the delicate parts inside the flower, and that the fungus soon penetrates the kernel, sapping its life, and forms new spores, which may spread to other flowers throughout the field. Atanasoff (*3*) also concluded that infection was local rather than systemic.

SYMPTOMS

Wheat scab, or head blight, is found throughout the wheat-growing areas of the Corn Belt. If conditions are favorable for its development and spread, the disease, by preventing the normal growth of infected kernels, may cause a marked reduction in yield; and, because of the metabolic processes of the fungus within the kernels, the grain produced will be of inferior quality. (Fig. 1, A.)

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[3] Reference is made by number (italic) to Literature Cited, p. 797.

The first symptom of infection within a spikelet may be a pinkish tint in the anthers protruding between the glumes, or a water-soaked area on a glume that first turns purplish brown and then loses its

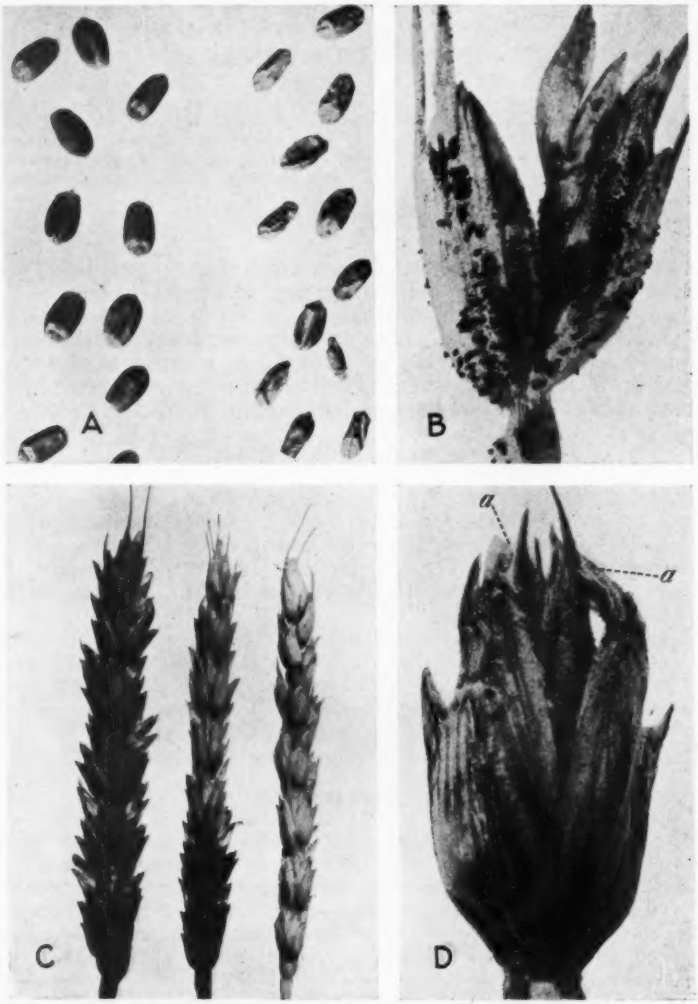

FIGURE 1.—A, Healthy kernels of wheat (left) and blighted or scabbed kernels (right); B, perithecia of *Gibberella saubinetii* on glumes and rachilla of infected spikelet of wheat; C, normal green head of wheat (left) and two infected heads (right), lower spikelets on middle head not diseased; D, infected spikelet containing caught anthers (*a*) and showing fungus growth on external surface of glumes

color. Later on, masses of conidia often give a pink color to the margins or surfaces of the glumes. (Fig. 1, D.) Small blue perithecia may occur on the glumes or at the base of spikelets, especially in

certain bearded winter wheats. (Fig. 1, B.) If only scattered spikelets are infected, their smaller size and bleached glumes form a marked contrast to the adjacent normal green spikelets. The first infection usually occurs near the middle of the head. Spikelets just above and below those first attacked often develop the disease, and finally the entire head may be killed, as indicated by the characteristic bleached-straw color. (Fig. 1, C.)

MATERIAL AND METHODS

The experiments reported in this paper were carried out in the field and in greenhouses at the University of Wisconsin, Madison, Wis. Inoculations were made almost exclusively on pure-line Marquis and Prelude wheats. In the field, infections were studied as they occurred naturally and as they resulted from inoculations with a conidial suspension of *Gibberella saubinetii* on wheat heads in various stages of development, kept at the necessary high humidity in glassine bags during the period of incubation. In the greenhouse, plants in flats were brought to flowering by the aid of artificial light, so that they were ready for inoculation in the early spring. After inoculation the plants were placed in chambers in which the conditions of temperature and humidity could be controlled. The chambers were kept at constant temperatures of approximately 12°, 20°, 24°, 28°, and 32° C. At 28° and 32° the relative humidity was maintained at 70 to 75 percent. At the lower temperatures the humidity was lowered until the rate of evaporation, as indicated by the Livingston atmometer, was the same as that maintained at the higher temperatures. Notes were taken on the progress of infection until the disease had run its course or the heads were harvested.

The stages of development of the wheat heads recorded in the tables were determined by observation at the time of inoculation. Individual spikelets were recorded as "flowering" if one or more flowers displayed anthers that were not fully discharged. When the anthers were white and appeared to contain no pollen the spikelet was considered to be in the "past-flowering, A" stage. The period from the time when anthers no longer adhered externally to the glumes to the soft-dough stage was termed the "past-flowering, B" period in the development of the kernel.

The greater part of the histological material studied was killed in Gilson's fixative[4] or in Juel's zinc-chloride fixative,[5] embedded in paraffin, sectioned, and stained in Delafield's haematoxylin and erythrosin. The difficulty usually encountered in cutting serial sections of entire spikelets was overcome by demineralization with hydrofluoric acid. After being killed and partly dehydrated, entire spikelets as well as the hard mature kernels were immersed for 72 hours in equal parts of hydrofluoric acid and 70 per cent alcohol. For spikelets in the flowering stage, 53 hours' treatment with the hydrofluoric acid solution proved sufficient. The material was then washed in water for 24 hours and put through the paraffin-embedding process. Szombathy's "tanning" process, as recommended by Artschwager (*9*), was employed in fixing the paraffin ribbons to the slides.

[4] Gilson's fixative: 95 per cent alcohol, 126 cc; distilled water, 180 cc; glacial acetic acid, 54 cc; concentrated nitric acid, 6 cc; mercuric chloride (saturated solution), 33 cc.
[5] Juel's fixative: zinc chloride, 10 g; glacial acetic acid, 10 g; 50 per cent alcohol, 500 cc.

FIELD AND GREENHOUSE STUDIES

INITIAL INFECTION AND INCUBATION PERIOD

In the greenhouse and in the field, studies were made on initial infection and length of incubation period in wheat scab as influenced by temperature, humidity, and the stage of development of the wheat head.

In the greenhouse in 1923 and 1924, inoculation of the heads was more effective at flowering time than at earlier stages, though under certain conditions there was a relatively high percentage of infection when the inoculum was applied in the out-of-the-boot and before-flowering stages. (Table 1.) In all cases it was necessary to maintain a high atmospheric humidity, without which infection is difficult. In the field, in 1924, inoculations made at flowering time or just previously gave the highest percentages of infected kernels per head. (Table 2.) Perhaps the best indication of the relation between the stage of development of the host and the severity of infection is the reduction in weight of infected kernels per head. Table 2 and Figure 2 show that the greatest reduction in weight of kernels occurred in heads inoculated at flowering time. The percentage of infection in both Marquis and Prelude wheat increased as the temperature rose. (Table 3.) At 12° C. infection occurred on extruded anthers but did not spread into the living tissues.

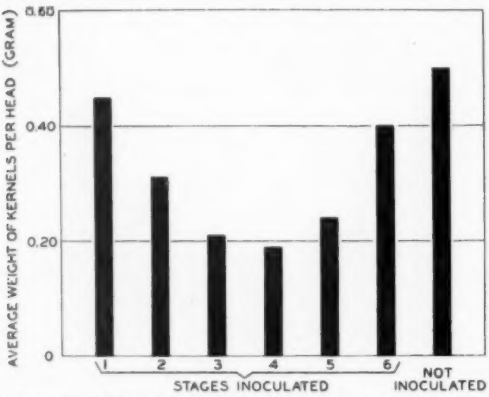

FIGURE 2.—Relation between stage of development in which heads were bagged and inoculated and the weight of grain per head. (Data in Table 2)

TABLE 1.—*Infection in heads of wheat inoculated at various stages of development with Gibberella saubinetii and incubated during the progress of infection at 20°, 28°, and 32° C.*

Stage of development at inoculation	Prelude wheat *			Marquis wheat		
	Heads inoculated	Heads infected		Heads inoculated	Heads infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
In boot	33	0	0	15	1	6.6
Half out of boot	13	0	0	17	1	5.8
Out of boot	14	0	0	51	31	60.7
Before flowering	52	14	26.9	100	55	55.0
Flowering	81	31	38.2	143	98	68.5

* Notes taken for one week only because of injury by fumigation.

TABLE 2.—*Average number of kernels, percentage of infected kernels, and percentage reduction in weight of kernels, per head, of Marquis wheat inoculated in the field at various stages of development with Gibberella saubinetii*

Stage of development at inoculation	Healthy-kernel data		Infected-kernel data			
	Heads counted	Average kernels per head	Heads counted	Infected kernels per head	Heads weighed	Average reduction in weight of kernels per head following inoculation
	Number	*Number*	*Number*	*Per cent*	*Number*	*Per cent*
1. In boot	7	18.8	2	7.98	5	11.1
2. Emerging from boot	17	21.2	7	22.2	11	37.7
3. Half out of boot to before flowering	71	17.1	45	56.6	26	58.4
4. Flowering	15	19.9	11	50.0	17	63.0
5. Past flowering, A	16	25.0	11	43.24	15	52.9
6. Past flowering, B	30	24.8	19	35.2	31	20.4
Control (not inoculated or bagged)	100	23.8	100	0	18	0

TABLE 3.—*Infection in heads of Prelude and Marquis wheat inoculated at various stages of development with Gibberella saubinetii and incubated at various temperatures during the development of the disease*

Temperature during incubation °C.	Prelude wheat			Marquis wheat		
	Heads inoculated	Heads infected		Heads inoculated	Heads infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
12				124	0	0
20	52	1	1.9	150	67	44.6
28	60	12	20.0	176	118	67.0
32	81	32	39.5			

In the greenhouse, the length of the incubation period was influenced by temperature, the higher temperatures accelerating the appearance of the disease. (Table 4.) The length of the incubation period varied also with the stage of development of the spikelet, decreasing as the plants matured, as shown by inoculations made July 16 to 25, 1924. (Table 5.) Although inoculum was applied in some instances as early as the out-of-the-boot stage, infection was not evident until the flowering stage. Such an interval implies either that infection may have occurred early but developed so slowly that it was not apparent until flowering time or that it actually did not occur before the flowering stage. Microscopic examination of sectioned material indicated that the latter supposition is correct. Moreover, under favorable conditions, the fungus may remain viable on the head for a number of days. In that case, the progress of infection on the wheat head might be expected to follow, to some extent at least, the progress of anthesis.

TABLE 4.—*Incubation periods at various temperatures after inoculation of heads of Marquis wheat, in the before-flowering and flowering stages, with Gibberella saubinetii*

Temperature °C.	Stage of development	Heads inoculated	Average length of incubation period	Temperature °C.	Stage of development	Heads inoculated	Average length of incubation period
		Number	*Days*			*Number*	*Days*
20	Before flowering	11	16.4	28	Flowering	16	8.1
20	Flowering	8	15.8	32	Before flowering	6	4.5
28	Before flowering	16	10.1	32	Flowering	21	4.5

TABLE 5.—*Incubation periods after inoculation, in the field, of heads of Marquis wheat, in various stages of development, with Gibberella saubinetii*

Stage of development	Heads inoculated	Average length of incubation period	Stage of development	Heads inoculated	Average length of incubation period
	Number	*Days*		*Number*	*Days*
Emerging from boot	18	° 12	Flowering	47	3.6
Half out of boot	40	6.9	Past flowering, A	25	° 5
Out of boot	35	6.6	Past flowering, B	35	3
Before flowering	28	4.1			

° Period probably too long. No reading for two days preceding the record of first infection.

ANTHESIS AND THE PROGRESS OF INFECTION

Concerning the interval between emergence from the boot and flowering, Percival says (*7, p. 122, 124*):

> Anthesis, or opening of the flower, follows the escape of the ear from the upper leaf-sheath in five or six days, although in some instances it occurs on the first day, or may be delayed until the ninth day after the appearance of the ear * * *. In ears possessing 16–18 spikelets the 8th to 12th usually open first; in those with 28–30, the 16th to 20th. Anthesis proceeds upwards and downwards from these points in more or less regular succession, the apical and basal spikelets being the last to flower.

According to Leighty and Sando (*5, p. 236*), it seems to be the consensus of opinion that the first spikelets to flower are situated in the upper part of the middle third of the head, or in the lower half of the upper third. They say that, considering the head as a whole, the flowers above the first flower to bloom complete their blossoming before those situated below this flower. In the spikelet, the lowest flower usually blooms first, followed by the others in order from lowest to highest on successive days. They also state (*5, p. 240*) that it seems evident—

> That temperature, rainfall, and sunshine are intimately associated with the blooming of wheat flowers * * *. Temperature, however, seems to be the most important of the meteorological influences affecting blooming.

In the experiments that they recorded, the maximum blooming occurred on May 15 and 16, days when the temperatures were 76° and 78° F., respectively, and when almost perfect sunshine with no rainfall was registered. Anthers in flowers opening on clear days dehisced more rapidly than those in flowers opening on cloudy days. They state that—

Cloudiness or rain has the effect of retarding blooming, principally through the lowering of the temperature to a point below the optimum for this process. On cloudy or rainy days, however, when the temperature is favorable for blooming the flowers either open incompletely or bloom cleistogamously. On May 18 flowers were observed opening in a drizzling rain and pollen was shed from the anthers. No flowers were observed to open at any time during a rain of such intensity as to permit droplets of water to adhere to the glumes.

In 1923, experiments on 27 heads of Marquis wheat inoculated before blossoming by spraying with a conidial suspension of *Gibberella saubinetii* and incubated under controlled conditions of temperature and humidity showed that the location of first infections paralleled somewhat the theoretical progress of anthesis, as illustrated in the following tabulation:

Spikelet No.[6]	Number of first infections[7]	Spikelet No.[6]	Number of first infections[7]
1	0	7	6
2	0	8	3
3	0	9	6
4	1	10	1
5	4	11	3
6	8	12	3

In these inoculations, 77.1 per cent of the first infections were recorded as occurring on spikelets 5 to 9. No record was made of the average number of spikelets per head. The heads were, however, shorter than those grown in the field, which averaged a little more than 16 spikelets per head.

In 1924, inoculations were made in the field on heads of Marquis wheat that were flowering. Individual spikelets of these heads were recorded as "before flowering", "flowering", and "after flowering". The record of first infections on 25 of these heads showed 27 per cent of the first infections in spikelets that had not yet reached the flowering stage at the time of inoculation, 53 per cent in those that were in the flowering stage, and 18 per cent in those that were in the past flowering stage at the time of inoculation.

In 1923, heads of Marquis wheat in the early-flowering stage were inoculated with a conidial suspension of *Gibberella saubinetii* and incubated at 20° and 28° C. The results (Table 6) show that all first infections occurred on spikelets in flower or on spikelets adjacent to them.

On July 18, 1924, heads of Marquis wheat in the early-flowering stage were inoculated in the field with a conidial suspension of *Gibberella saubinetii*. The results (Table 7), with two exceptions, were similar to those obtained in the preceding experiment.

On July 19, 1924, heads of Marquis wheat in the flowering and past-flowering stages were inoculated in the field with a conidial suspension of *Gibberella saubinetii*. The results (Table 8) show that the position of first infection was less regular than in the two previous experiments. Because of the difficulty of determining the later stages in the development of the spikelets, errors are more apt to occur than in records of the earlier stages. Nevertheless, Table 8 indicates that first infections follow in some measure the progress of anthesis, since the larger percentage of first infections on the

[6] Spikelets are numbered from base of head upward.
[7] In some cases 2 adjoining spikelets on a head were found infected at the first reading.

lower spikelets occurred on the more mature heads. In Table 8 the percentage of infections occurring below spikelet 7 is 42.3; in Table 7, 20.

It is difficult to compare the progress of infection on wheat heads with the progress of anthesis because both are often irregular. Apparently there are numerous exceptions to the general order of progression in flowering upward and downward from some spikelet in the upper part of the middle third of the head. According to Percival (*7, p. 124*), "Sometimes 4 or 5 spikelets on one side open before the rest; in other cases adjacent spikelets on opposite sides of the rachis flower almost simultaneously." Data on the progress of infection by spikelets on heads inoculated before blossoming and incubated at 20° and 28° C. are given in Tables 9 and 10. It will be noted that in some cases the progress of infection seems to parallel the theoretical progress of anthesis. In other cases the relationship is not so evident.

In order to learn the trend of infection, two composite heads, A and B, were constructed from Tables 9 and 10, respectively. The resulting summaries are given in Table 11.

In head A infection first appeared in spikelet 7 and spread upward and downward, the basal spikelet being the last to show infection. The time between the appearance of the first infection and the infection of the basal spikelet was 8.5 days.

In head B infection first appeared in spikelet 9, and in 4 days spread upward to spikelet 15 and downward to spikelet 4. Heads 362 and 410, of Table 10, were irregular from the standpoint of the theoretical progress of anthesis. As a result, infection appeared in the first three spikelets of head B, before it appeared in spikelet 4. Otherwise the progress of infection upward and downward from the initial infection followed the theoretical order of anthesis. At 28° C. the time between the first infection and the last infection on head B was 4 days. This period, compared with the 8.5 days at 20° required to complete the spread of infection in head A, corresponds with the probable difference in the rate of flowering at the two temperatures. Concerning the time required for flowering, Percival (*7, p. 124, 128*) says:

> At Reading the whole ear often completes its flowering in 3 to 5 days when the air is warm and the sky clear; in wet or dull weather the period is prolonged to 6 or 8 days.

In view of the possibility that infection may proceed through the rachis from the spikelets first infected to adjoining spikelets, a study was made of serial sections of the rachis on either side of visibly infected spikelets and also of serial sections of apparently clean spikelets adjoining spikelets visibly infected at the time of fixation. Mycelium was found to have advanced from infected spikelets through the rachis for some distance in both directions, but in the sections examined it had not actually invaded the adjoining spikelets. Evidently this rachis infection usually resulted in cutting off the food and water supply of spikelets as well as in producing toxic by-products, thus bleaching the spikelets and shriveling the kernels. These symptoms may easily be confused with those produced by the presence of mycelium within the spikelets. Often the real condition can be determined only by plating the kernels, or by microscopic ex-

amination of sectioned material. Undoubtedly some of the spikelets recorded as infected in Tables 7, 8, 9, 10, and 11 may be of this type. It is also conceivable that long intervals between initial infection and infection of neighboring spikelets may indicate infection through the rachis rather than from the exterior.

TABLE 6.—*Position of spikelets showing first infections in heads of Marquis wheat inoculated in the early-flowering stage with Gibberella saubinetii and incubated at 20° and 28° C.*

[F denotes flowering stage; asterisk (*), first infection]

Temperature (° C.)	Head No.	Spikelet Nos.[a] flowering when inoculated and those showing first infections														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
20	360					F		F		F	F	F*				
20	361					F*	F	F	F	F*	F	F	F	F		
20	465						F*	F*	F*							
20	468							F	F	F	F*					
20	471				F	F*	F	F	F	F	F	F	F	F		
28	365				F	F	F	F	F	F	F	F*	F			
28	368				F	F	F	F	F*	F	F	F	F			
28	369						F*									
28	433					F	F	F	F	F	F	F	(*)			
28	439				F	F	F	F*	F	F	F	F	F	F	F	
28	440					F*	F	F	F	F	F	F	F	F	F	F
28	445						F	F	F	F		F*				
28	450						F	F	F	F*	F	F	F	F		
28	451						F	F	F	F	F	F	F*			
28	460					(*)	F	F	F	F	F	F*	(*)			

[a] Spikelets are numbered from base of head upward.

TABLE 7.—*Position of spikelets showing first infections in heads of Marquis wheat inoculated in the field, in the early-flowering stage, with Gibberella saubinetii*

[F denotes flowering stage; asterisk (*), first infection]

Head No.	Spikelet Nos.[a] flowering when inoculated and those showing first infections														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
584								F*	F	F	F	F	(*)		
586							F	F	F	F	F*	F			
587							F	F	F	F	F	F	(*)		
588							F	F	F	F	(*)				
589								F*	F	F	F	F			
590						F*	F*	F	F*	F	F				
591					(*)	(*)	F	F	F	F	F				
592						F	F	F*	F		F				
593						(*)		F	F	F	F	F	F		
594							(*)	F*	F	F*	F	F			
595							F	F*	F	F*					
596								F*	F						
598						(*)	F	F*	F	F	F	F	F		
599							F	F*	F	F	F	(*)			
601							(*)	F	F	F	F	F			
602							(*)	F*	F	F		(*)			
603			(*)					F	F	F	F	F			
604							F	F*	F	F					
605							F*	F*	F*	F	F				
606								F*	F	F	F				
607					F	F	F	F*	F	F	F	F	F		
608								F		F	F*				
609						F*	F	F	F	F	F	(*)			
610			(*)					F	F	F					
611							F	F*	F	F*					

[a] Spikelets are numbered from base of head upward.

TABLE 8.—*Position of spikelets showing first infections in heads of Marquis wheat containing spikelets in the before-flowering, flowering, and past-flowering stages when inoculated in the field with Gibberella saubinetii*

[F denotes flowering stage; P, past-flowering stage; asterisk (*), first infection]

Head No.	Spikelet Nos.[a] flowering and past flowering when inoculated and those showing first infections																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
622			F				P	P*	F	P		P	P	F	P	F	P
624					F	F	P*	P	P	P	P	P					
626					F	F*	F	F	F*	F	F	F		P			
628						F*	F	F		F	P		F	F	F	F	F
630						F	P	F	P*	P*	P	P		(*)	F		
632						F	P*	F	P	P	P	F					
633				(*)			F	F	F								
634				(*)	P	P		P	P	P	P	P	P	F			
636				F*	P*	P	P*	P	P	P	P	P	F	P	P	P	
637				P	P	P	P*	P	P	P	P	P					
638				F		F*	P	P*	P	P*	P	P	F		P		
639			(*)	(*)		P	P	P	P			P	P				
640						F	F	P	P*	F	F	P	P	F	P		
641						F	F	F*	F	P	P	F	F				
642				(*)	F	F*	F	F*	P	P*	P	P	F	P			

[a] Spikelets are numbered from base of head upward.

TABLE 9.—*Progress of infection by spikelets on heads of Marquis wheat inoculated before flowering with Gibberella saubinetii and incubated at 20° C.*

[Asterisk (*) denotes first infection]

Stage of development when inoculated	Head No.	Position of first infection and interval (days) between first and later infections for spikelet No.[a]—												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Out of boot	388						4	4	2	(*)	2	4		
	391					9	9	8	8	8	8	(*)		
	398	8	8	8	7	6	(*)	7						
	399	5	5	5	4	5	(*)	5						
	406	2	2	2	(*)	(*)								
Before flowering	493	8	8	8	7	7	5	(*)	5	7	7	7	7	
	498			2	2	(*)	2	2						

[a] Spikelets are numbered from base of head upward.

TABLE 10.—*Progress of infection by spikelets on heads of Marquis wheat inoculated in the out-of-the-boot stage with Gibberella saubinetii and incubated at 28° C.*

[Asterisk (*) denotes first infection]

Head No.	Position of first infections and interval (days) between first and later infections for spikelet No.[a]—														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
362					2				3	2	(*)	(*)			
366						(*)		1							
367						2	2	(*)	(*)	1	2	2	2		
385				5	5	4	1	3	(*)	3	3	3			
410	2	2	2	2	1					2	1	(*)			
411	4	4	4	3	(*)	2	3				4				
413	3	3	3	3	1	(*)	1			3					
428						4	3	3	2	2	(*)	(*)	3		
429							2	2	(*)						
430					3	3	(*)	3	1	3	3	3			

[a] Spikelets are numbered from base of head upward.

TABLE 11.—*Progress of infection by spikelets on two composite heads [a] of Marquis wheat*

[Asterisk (*) denotes first infection]

Composite head	Temperature (° C.)	Position of first infections and interval (in days) between first and later infections for spikelet No. [b]—														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	20	8.5	7.5	7.2	5.7	4.9	4.3	(*)	4.2	4.2	7	7	7	----	----	----
B	28	2	1	2	4	3.8	3.5	2.4	2.1	(*)	2.2	2.0	2.7	2.7	3	4

[a] Method of construction of composite head A: The position of the spikelet in which first infection would occur in the composite head was determined by multiplying the number of times first infection occurred on each spikelet by the position number of that spikelet; the sum of these products divided by the total number of first infections gave the location of initial infection in the composite head as 6.63, or spikelet 7. The heads in Table 9 were then shifted in such a manner that all first infections fell in the seventh spikelet position, and the mean interval of time between the first and later infections was determined for each spikelet position.

[b] Spikelets are numbered from base of head upward.

A possible explanation of the occurrence of infection at blossoming time was suggested by Atanasoff (*4, p. 90*). He says that "Fusaria can not infect the cereal crops before the plants have passed the blossoming period, i. e., before they cease rapid development and cell division." It is characteristic of weak parasites that they are more destructive when the host is growing under unfavorable conditions. Adams (*1, p. 116–117*) mentions this point in relation to head blight and cites several examples. In the present investigation the plants were kept under as favorable growing conditions as possible, except that in some cases in the greenhouse unfavorable temperatures were used. At the higher temperatures the percentage of infection reached its maximum. With the exception of the single head of Prelude wheat found infected at 20° C., which was recorded as inoculated before flowering, the highest percentage of infected heads at all temperatures was found in the groups that were flowering when inoculated. The low percentages of infection in Prelude wheat as compared with Marquis (Table 1) are due, partly at least, to the fact that notes on Prelude were taken for only 1 week after inoculation, whereas notes were taken on Marquis until the plants were dead, and also to the fact that Prelude is more resistant than Marquis, as indicated by later experiments.

No infection occurred at 12° C., a temperature decidedly unfavorable for the rapid development, pollination, and fertilization of wheat plants when in the flowering stage. Threshing notes show that practically no kernels had been set on plants grown at that temperature. Leighty and Sando (*5, p. 242*) report that flowers were not seen to open at a temperature below 56° F. (13° C.), but that pollen was observed to be discharged at 52° F. (11° C.). Authorities cited by them give 12°, 13°, 14°, and 16° C. as the minimum temperatures at which flowers were observed to open. Some of the plants used in these experiments were flowering when they were inoculated and placed in the 12° chamber, so that certain spikelets may be considered to have flowered normally. Nevertheless infection failed to occur, probably because of the effect of an unfavorable temperature on both host and fungus, and possibly because of a lack of adequate humidity in the low-temperature chamber.

It seems clear that infection does not usually occur first on the outside of the glumes. Only one spikelet of the many examined showed the parasite growing on the outside of the enveloping glumes. In that case it appeared to be growing in a drop of water lodged on the surface and had not actually penetrated the glume. No fungus was found within the spikelet. Inoculation experiments in the field in 1924 tend to confirm the belief that infection does not originate on the outside of the glumes. Areas on the outside near the center of 30 glumes on spikelets in the before-flowering to the just-past-flowering stages were marked on 18 heads with a ring of india ink and then inoculated by applying a drop of spore suspension in sterile water, or a bit of infected agar, or by pricking with a needle dipped in spore suspension. The material was fixed at intervals of from 1 to 9 days. Macroscopic observations indicated that no infection had occurred except in the case of the needle-prick inoculations, although conditions of humidity and temperature were favorable for rapid infection. The prick inoculations were made in a cool period with minimum and maximum temperatures of 14° and 18°, respectively, at which the rate of infection is usually slow. Of the 9 glumes cut, only 2 showed penetration by the fungus. In one of these the hyphae had not advanced beyond the cells injured by the needle prick; in the other, hyphae were found to have penetrated to the depth of a few cells at the base of a spikelet inoculated with infected agar.

Further indication that initial infection does not occur on the outside of the glumes was found upon examination of serial sections of a number of diseased spikelets. Although the kernels showed a network of mycelium, there was practically no fungus on the outside of the glumes.[8] When infection of a spikelet has developed sufficiently to attract the attention of a casual observer, there are often sporodochia at the base and along the margins of the outer glumes. The possibility that conidia may be held in drops of water at the base of the spikelet until invasion has been effected is recognized, although it has not been observed to occur in any of the numerous cases of initial infection studied. Growth of the fungus on the exterior seems to follow infection within the glumes.

After anthesis, infection appears to occur first on degenerating tissues such as anthers, styles (fig. 3, A and B), and pericarp, and to penetrate from these into the developing kernel or the inner surfaces of the glumes.

Caught and retained anthers were the first tissues observed to serve as a base for invasion into the developing kernel. In field inoculations during 1920 it was noted that anthers caught between glumes frequently showed the first sign of infection. Within a short time the glume from which such an anther extended developed a water-soaked and discolored area. By fixing and sectioning spikelets in the very early stages of infection, clear cases of infection beginning in caught and held anthers were found.

Little information has been found in the literature on the frequency of retained anthers. Leighty and Sando (*5, p. 233*) noted that 5 percent of the 406 flowers on the heads under observation did not open at all or opened only far enough to allow the tips of the anthers to

[8] The term "glume" is here construed in the broader sense to include lemma and palet.

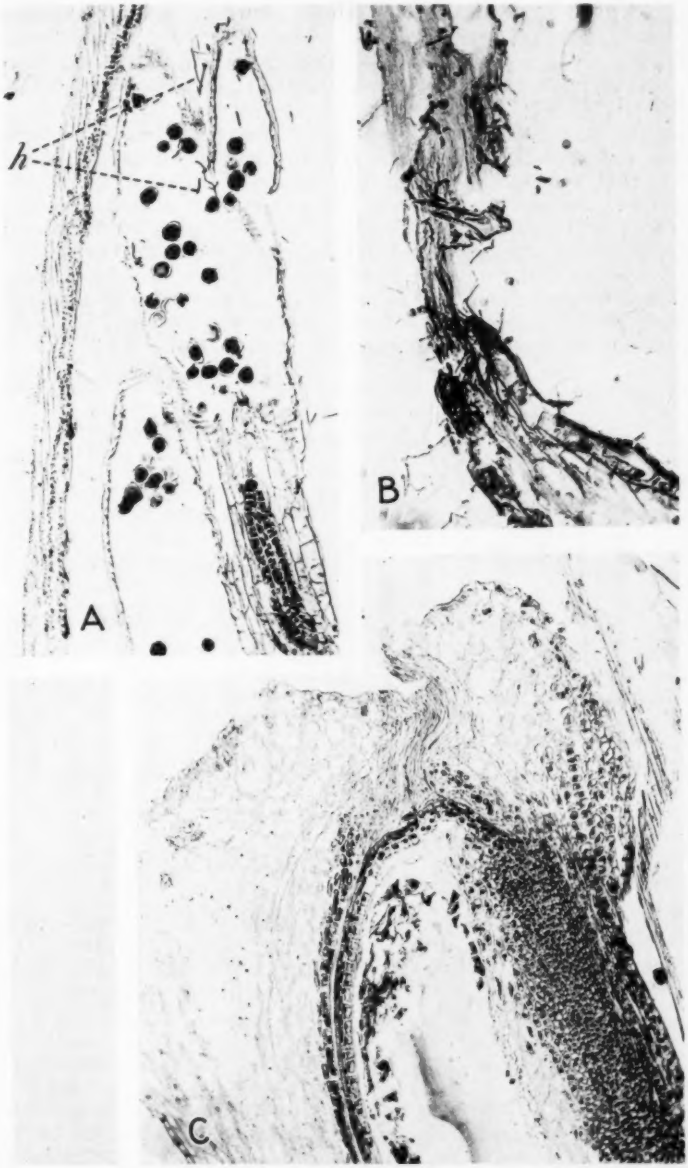

FIGURE 3.—A, Portion of a longitudinal section of a spikelet showing an early stage of anther infection; *h*, hyphae. × 57. B, Portion of a degenerating style which apparently served as a connecting tissue between an infected anther and the kernel in the same flower. × 136. C, Portion of a longitudinal section of the developing kernel in the flower containing the anther in A; serial sections show that there is no fungus in the kernel. × 57

extrude between the glumes, but that all these flowers set kernels. Percival (*7, p. 124*) says:

> Retention of the anthers, which is most frequent in dense-eared wheats, occurs chiefly in the lower spikelets of ears and the smaller flowers of each spikelet, some of which do not open at all.

In the season of 1924 records were made of retained anthers in inoculated heads and also in the 100 uninoculated heads of Marquis wheat that were harvested for the purpose of obtaining information on caught anthers, independent of inoculation. Notes were taken only on the first and second flowers, for it was found upon examination of 15 heads of uninoculated Marquis wheat grown in the field that only 6 per cent of the third flowers in the spikelets had set kernels. The data presented in Table 12 show that there were retained anthers in 22.5 to 85.5 per cent of the fertile flowers observed and in 58 to 92 per cent of the spikelets counted. They also indicate that retention of

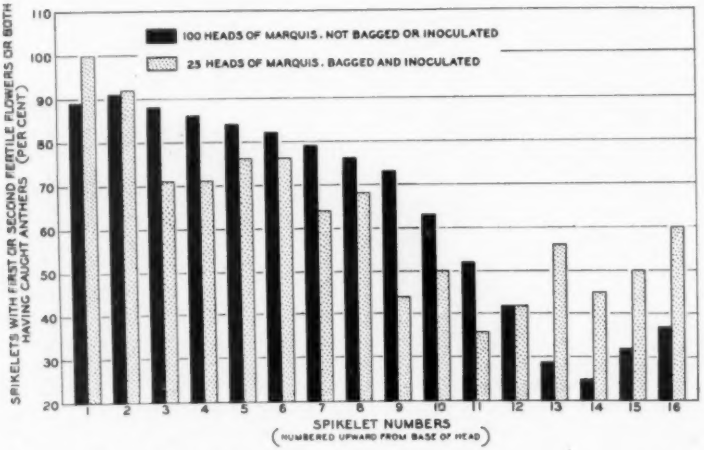

FIGURE 4.—Distribution of spikelets in Marquis wheat having one or both fertile flowers with caught anthers, based on 100 heads unbagged and uninoculated and 25 heads bagged and inoculated with *Gibberella saubinetii* in the field. (Data in Table 12)

anthers is not the result of infection, because the percentages for checks were higher than for inoculated plants. The data for Marquis wheat are presented graphically in Figure 4. It will be noted in Figure 4 that a comparatively high percentage of retained anthers was found in spikelets Nos. 2 to 12, inclusive. The tabulation on page 777 and Tables 6 to 11 likewise show that, with three exceptions, all the first infections recorded occurred below spikelet 13. Two varieties of wheat studied, Norka (C. I. No. 4377[9]) and Carina (C. I. No. 3756) showed marked differences in the percentage of fertile flowers with retained anthers. (Table 13.) Likewise there was a higher percentage of infected kernels in the variety having the higher percentage of retained anthers.

[9] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

TABLE 12.—*Percentage of anthers held by glumes after anthesis in several varieties of wheat, uninoculated or inoculated, artificially or naturally, with Gibberella saubinetii, in the field*

Variety	Treatment	Heads observed	Fertile flowers with retained anthers	Spikelets with retained anthers in first or second or both fertile flowers
		Number	*Per cent*	*Per cent*
Marquis	Not inoculated	100	46.8	60
	Inoculated	25		58
Several varieties	Natural infection	6	52	
	do	15	34.5	
	do	8	22.5	
Norka (C. I. No. 4377)	Not inoculated	15	55.7	76.7
	Inoculated	18	47	
Carina (C. I. No. 3756)	Not inoculated	15	85.5	91.9
	Inoculated	11	73	
Variety not known	Not inoculated	15	69.8	83.4
	Inoculated	14	57.8	

TABLE 13.—*Percentage of fertile flowers with retained anthers and of kernels infected in Norka and Carina wheat inoculated in the field, in flowering to past-flowering stages, with Gibberella saubinetii*

Variety	C. I. No.	Heads examined	Fertile flowers with retained anthers	Kernels infected
		Number	*Per cent*	*Per cent*
Norka	4377	18	47	* 19.5
Carina	3756	11	73	40.1

* Percentage based on 17 heads.

HISTOLOGICAL STUDIES

In connection with the experiments in both field and greenhouse, histological studies of inoculated spikelets were made in order to determine the mode and course of infection in more detail than was possible from macroscopic observations alone. By means of serial sections through entire spikelets it was possible to follow the course of infection in the various parts of individual spikelets and to ascertain the presence or absence of retained anther tissue.

INFECTION OF GLUMES

It seemed clear from macroscopic observation of diseased spikelets that it is unusual for infection to begin on the outside of the glumes and then proceed to the kernel. Moreover, the inoculation of the outer surface of the glumes, in the field in 1924, as stated previously, gave negative results.

As long as the flowers of a spikelet remain closed the spikelet appears to be effectively protected against infection by the thickened and suberized walls of the outer epidermis of the glumes. This protection is found in the highest degree in the outer epidermis of the outer glumes and to a lesser degree in the outer epidermis of the lemmas and palets. The walls of the inner epidermis of the empty

glumes and of both flowering glumes were thickened toward the distal ends, the thickening extending considerably farther toward the base of the spikelet in the empty glumes than in the flowering glumes. As a rule, the walls of the epidermal cells of the glumes are thickened where they are exposed and are practically impenetrable. (Fig. 5, A.) Penetration of the less thickened walls of the inner epidermis of the glumes is not so difficult and apparently occurs frequently. (Fig. 5, B.)

The heaviest growth of mycelium in badly infected glumes, rachis, or culms is found in the chlorophyllous tissue. (Figs. 5, C, and 6, C.) Apparently the nutrient value of these cells is high and their thin walls offer little resistance to the fungus.

Sporodochia are often observed at the base of spikelets in advanced stages of infection. The stroma supporting them seems to be in the chlorophyllous tissue. Conidiophores emerge through the stomata at first (fig. 6, D), but with the massing of mycelium within the tissue they break through the epidermis elsewhere, frequently pushing outward between the lateral walls of the epidermal cells. (Fig. 6, A and B.) Sporodochia may extend upward from the base of the glumes, particularly along their lateral edges, but this growth also appears to follow internal infection. (Fig. 6, C and D.)

The vascular elements are not particularly attractive to Gibberella, although any bundle that lies in the path of the hyphae may be invaded. (Fig. 7, B.) The phloem shows much heavier infection than the xylem vessels. The thin cellulose walls of the phloem and companion cells are apparently penetrated without difficulty, and it seems reasonable to suppose that a rich medium is offered. There appears to be less fungus in the annular and spiral vessels than in the pitted ones. A rather uniform constriction of the hyphae as they pass through the walls of the pitted vessels indicates that passage is effected through the pits. The manner of penetration of the walls of the annular and spiral vessels was not determined. It seems probable that penetration is effected at some unthickened part of the wall.

In thoroughly infected glumes, mycelium may be found throughout the thick-walled cells of the ground parenchyma, but, owing to the thick lignified cell walls and the absence of cytoplasm, it is not massed as it is in the assimilating tissue. (Fig. 7, D.) These thick-walled lignified cells do not collapse even when heavily infected. The fungus apparently makes its way from cell to cell through the pits and not by penetrating any of the lignified parts.

The thin-walled ground parenchyma of the rachis and culm is composed of short-lived cells that are polygonal or rounded in cross section and have finely pitted walls. In this tissue the hyphae are found chiefly in the intercellular spaces. (Fig. 7, A.) Infrequently, the hyphae go directly through the cell walls, but the intercellular path, being the line of least resistance, is usually followed. In general it may be said that *Gibberella saubinetii* is both intercellular and intracellular in the various tissues of the host. The path of the fungus as it goes from cell to cell leads through pits or thin places in the walls. The attenuation of the hyphae to a threadlike thinness as they pass through the walls seems to be of common occurrence in all tissues. (Fig. 7, C.)

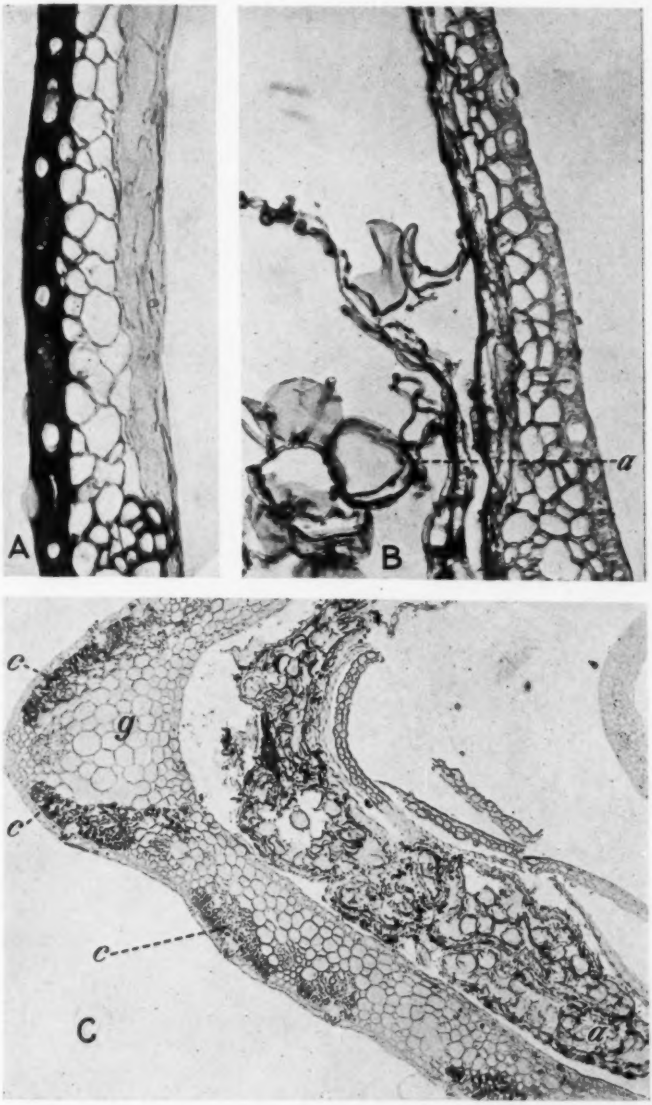

FIGURE 5.—A, Portion of a cross section of a lemma in an infected spikelet, stained with safranine and orange G to bring out differences in the nature and thickness of the walls of the epidermis on the outer and inner surfaces of the glume; the small amount of fungus present in the collapsed cells of the parenchyma is not brought out by these stains. × 379. B, Portion of another cross section of the spikelet shown in A, stained with safranine, Delafield's haematoxylin, and orange G to show the advance of fungus hyphae from an infected anther (*a*) into the lemma. × 265. C, Portion of a cross section of a spikelet, stained with Delafield's haematoxylin and erythrosin, showing location of chlorophyllous tissue (*c*) in an outer glume (*g*); caught anther (*a*) thoroughly permeated by hyphae; little if any fungus in glume. × 55

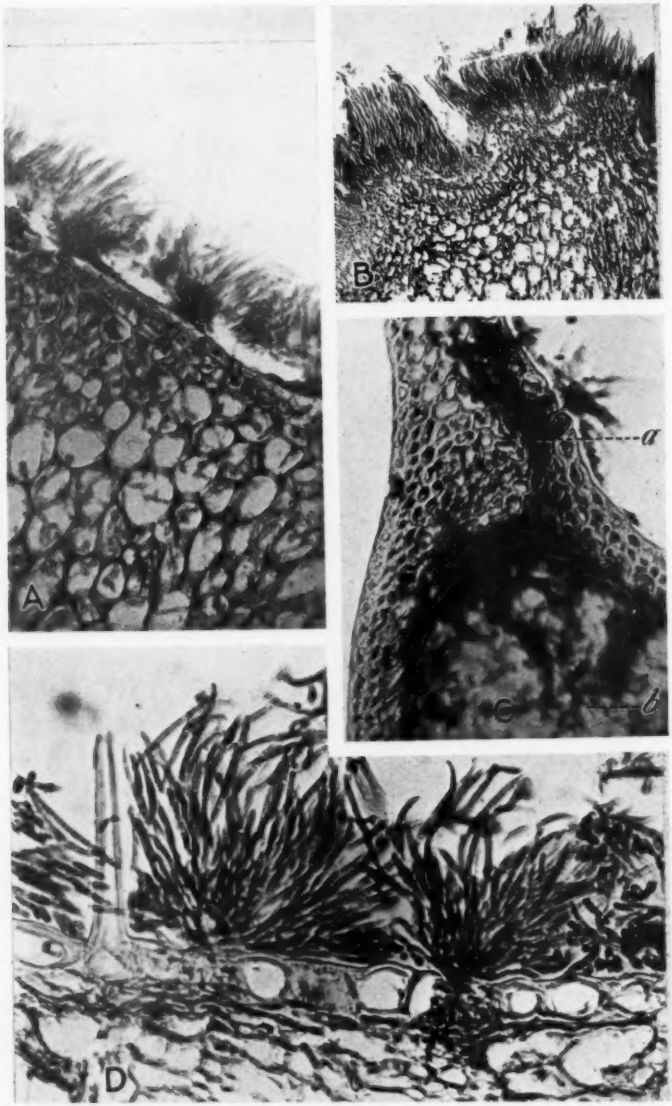

FIGURE 6.—A, Portion of a cross section through the rachis at the base of an outer glume, stained with Delafield's haematoxylin and erythrosin, showing hyphae emerging between cells of the epidermis and sporodochia on the surface. × 91. B, Sporodochia at the base of a glume, stained with Delafield's haematoxylin and erythrosin; the fungus is well established in the chlorophyllous tissue and has emerged through the stomata. × 194. C, Portion of a cross section of an infected palet showing entrance of the fungus through the inner epidermis, massing of hyphae in the chlorophyllous cells (*a*), and emergence through a stoma in the outer epidermis; the space (*b*) between the palet and kernel is webbed with hyphae. × 194. D, Emergence of hyphae through the stomata and formation of sporodochia on the outer surface of the glume, stained with Delafield's haematoxylin and erythrosin. × 347

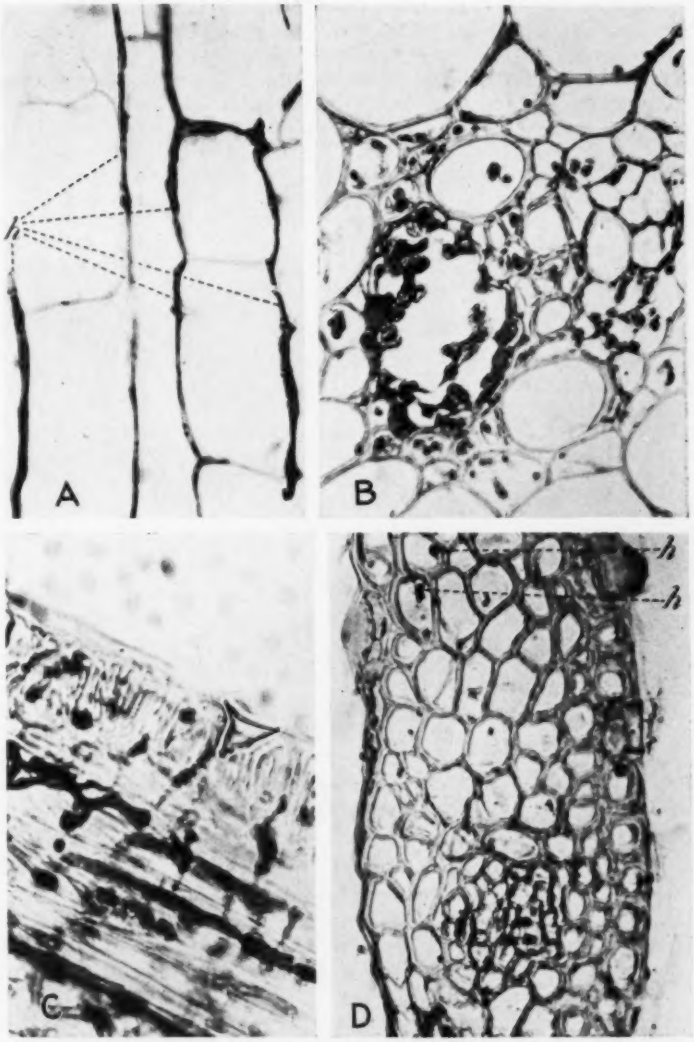

FIGURE 7.—A, Portion of a longitudinal section through the parenchyma of an infected rachis, showing intercellular path followed by the Gibberella hyphae. About × 305. B, Portion of a cross section of an infected rachis, showing hyphae within the vascular bundle. × 420. C, Portion of a longitudinal section of a lemma, showing the threadlike constrictions of the hyphae as they pass from cell to cell. × 612. D, Portion of a lemma showing occasional hyphae (*h*) within the cells of the thick-walled ground parenchyma. × 436. All sections stained with Delafield's haematoxylin and erythrosin

INFECTION OF ANTHERS

From a microscopic examination of fixed and sectioned material, it seemed evident that infection frequently begins in caught anthers. A number of spikelets fixed in the early stages of infection showed mycelium only within caught anther tissue. Others showed hyphae extending from infected anthers to adjacent parts of the flower. Of the material fixed and sectioned in 1920, 70 per cent of the 46 inoculated pieces either included infected anther tissue or contained no anther tissue and were not infected. Of the 1923 material, 29 of the 37 spikelets, or 78 per cent, of Prelude and Marquis wheat selected from the greenhouse inoculation experiments included either first or second flowers, or both first and second flowers, with retained anthers. Few cases of early infection were obtained in 1923. Serial sections of 8 spikelets, recorded at the time they were fixed as probable examples of infection originating in retained anthers, showed anther tissue in all, but only 4 were fixed early enough to show that invasion began in the anthers. (Fig. 3, A and C.) Anthers removed from a flower, inoculated with *Gibberella saubinetii*, incubated in a Van Tiegham cell at room temperature, and fixed 17 hours later, were found to be completely interlaced with the fungus hyphae (Fig. 8, B.)

Six good examples of initial infection through caught anthers, following the usual method of inoculation, were found among the 1924 spikelets sectioned. Additional information on this point was obtained in a special inoculation experiment in the field, in 1924, on the extruded anthers of 11 marked spikelets on 3 heads of Marquis wheat in the just-past-flowering stage. The extruded anthers were inoculated with a spore suspension of *Gibberella saubinetii* on July 30 and fixed on August 11. At the time of fixation, 8 of the spikelets appeared to be badly infected and 3 showed no symptoms of infection. None of the uninoculated spikelets showed infection. Two of the visibly infected spikelets were fixed and cut. Both contained caught anthers and kernels badly infected. It does not follow, however, that because one kernel in a spikelet is infected all are diseased. (Figs. 8 A, and 9, A–D.) Figure 10, A and B, shows cross sections through an infected anther of the second flower of a spikelet of Prelude wheat inoculated at flowering and fixed after an incubation period of 45 hours at 32° C. The serial sections show that the anther in contact with the kernel at the brush end is permeated by fungus and that hyphae have advanced into the pericarp of the second kernel. Toward the embryo end of this kernel the amount of fungus is scant. Hyphae have also penetrated the inner surfaces of the lemma and palet of the second flower, the outer surfaces in each case being clean, for the thickened and cutinized walls of the epidermis on the outer surface of the glumes render penetration difficult. Serial cross sections also show that this spikelet contains a basal and a third kernel, neither of which is infected. In the third flower there is a bit of infected caught-anther tissue, but hyphae had not reached any other part of the flower at the time the spikelet was fixed.

When infection occurs at flowering time, the fungus may easily penetrate the ovary. Shortly after anthesis the parenchyma of the pericarp begins to break down, the nuclei and cytoplasm of the cells disappear, the transition starch is moved into the developing seed, and the walls of the cells finally break down and are crushed together

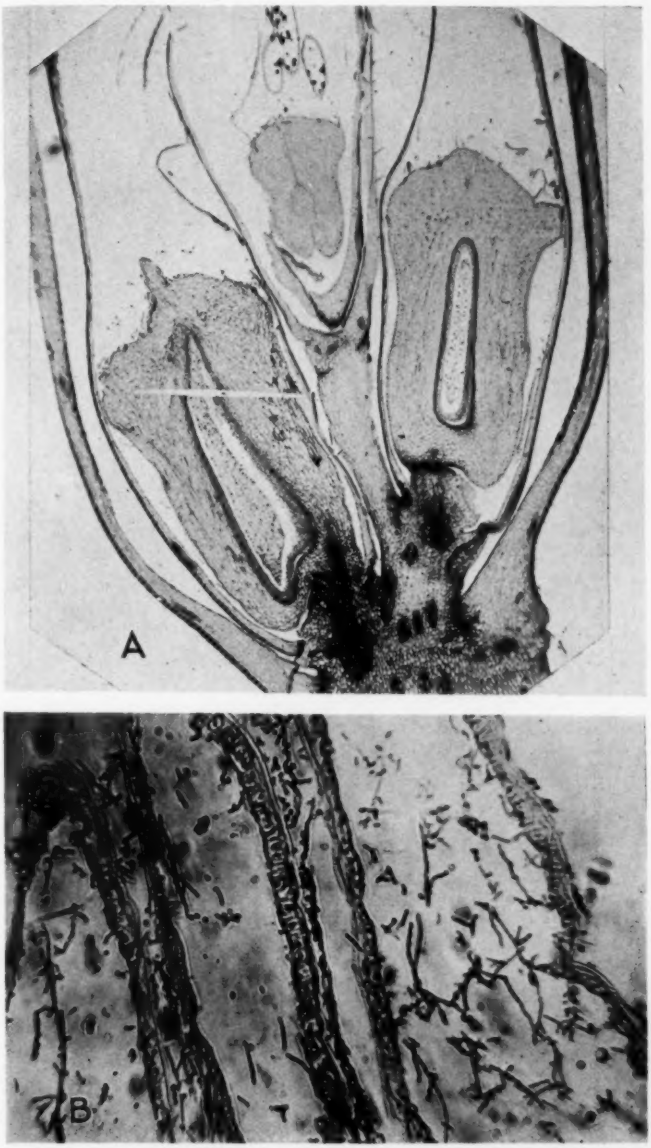

FIGURE 8.—A, Portion of a longitudinal section through an infected wheat spikelet. Serial sections of this spikelet show that the basal flower contains an infected caught anther and an infected kernel; the second flower is without a retained anther and the kernel is not infected; neither the anther tissue nor the kernel of the third flower is infected. × 16. (Details shown in fig. 9.) B, Portion of a section of an anther inoculated with *Gibberella saubinetii* and fixed after 17 hours' incubation in a Van Tieghem cell. × 142. Stained with Delafield's haematoxylin and erythrosin

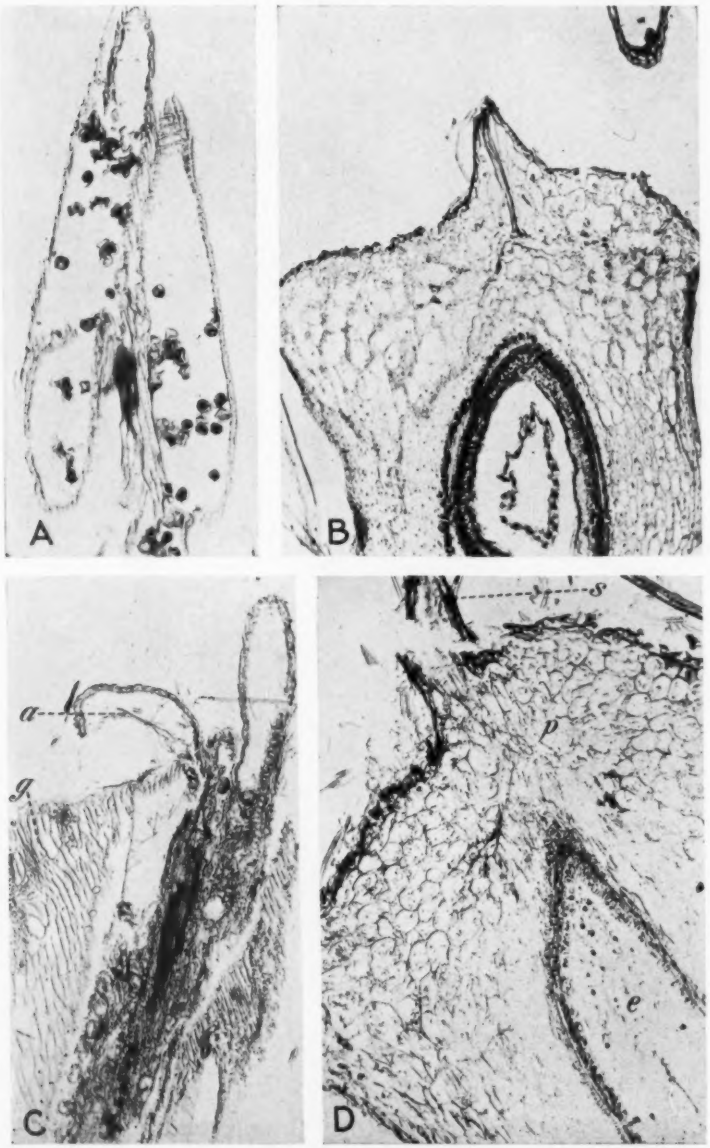

FIGURE 9.—Portions of a longitudinal section through an infected spikelet. A, Uninfected anther retained in the third flower. × 46. B, Developing kernel in the third flower. Serial sections show no hyphae in this kernel. × 46. C, Infected caught anther (*a*) in the basal flower of the spikelet; *g*, Glume. × 50. D, Infected basal kernel; *p*, pericarp (ovary); *e*, endosperm; *s*, remains of the style. × 50. Stained with Delafield's haematoxylin and erythrosin. (Another section through this spikelet is shown in fig. 8)

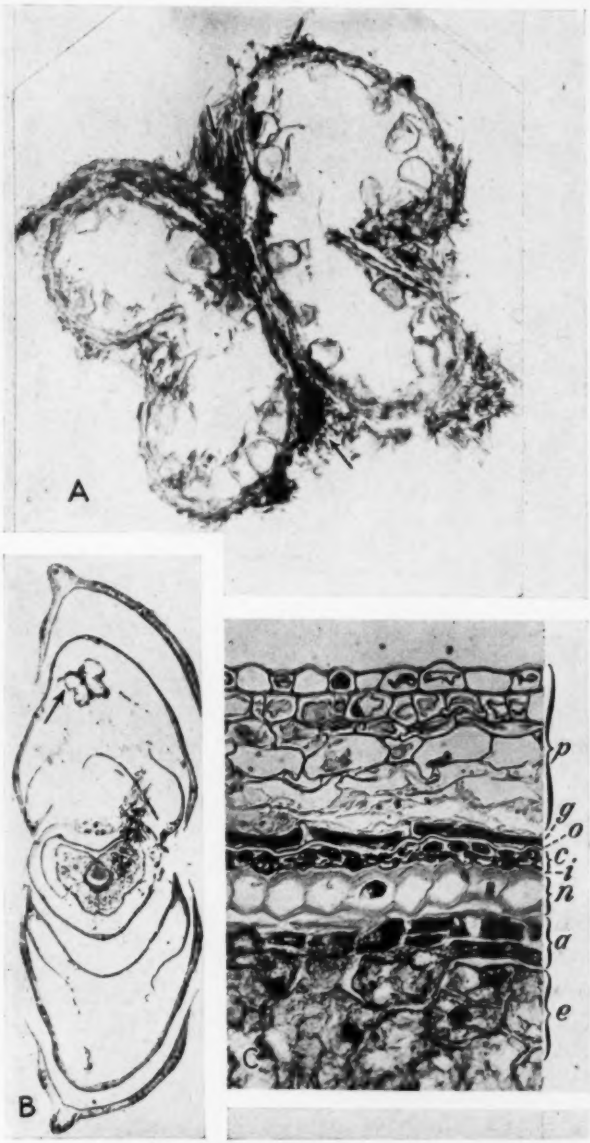

FIGURE 10.—A, Detail of anther, shown in B. Sporodochia containing conidia are indicated by arrows. × 132. B, Cross section through the upper part of a spikelet, showing the presence of an infected anther within the glume. × 12. C, Cross section of the outer layers of a developing kernel: *p*, Pericarp; *g*, chlorophyll layer (cross layer); *o*, outer layer of the testa (semipermeable membrane); *c*, color layer of testa; *i*, inner membrane of testa; *n*, epidermis of nucellus; *a*, aleurone cells forming; *e*, endosperm. × 264. Stained with Delafield's haematoxylin and erythrosin

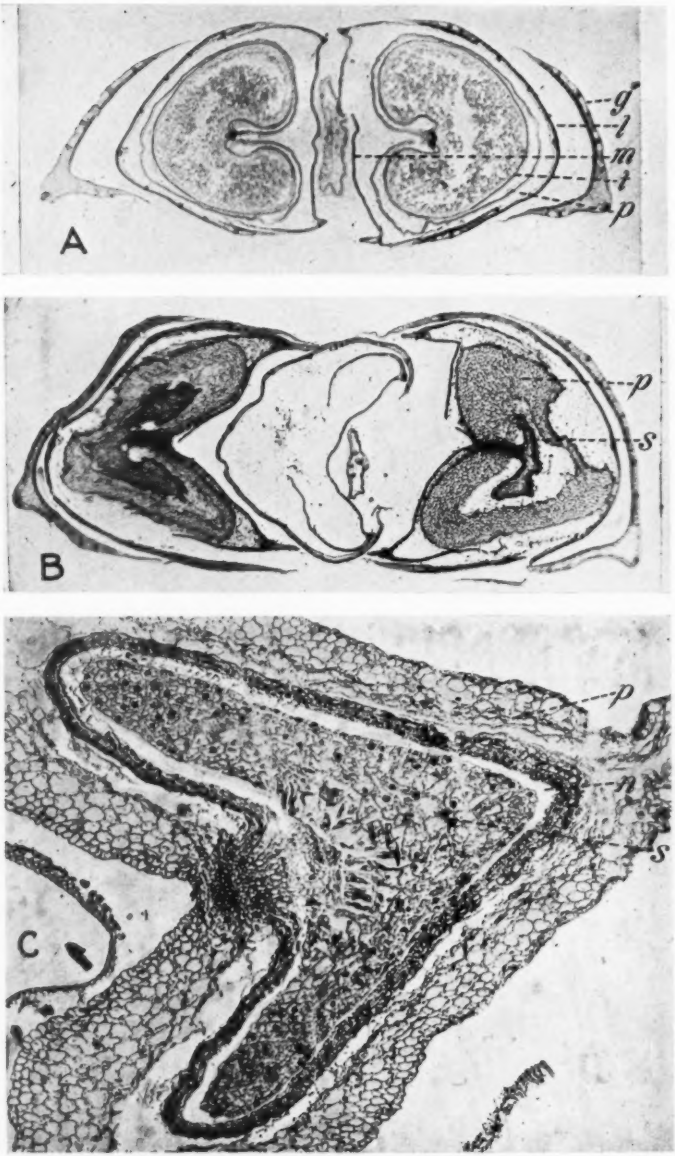

FIGURE 11.—A, Cross section of a nearly mature uninfected spikelet of wheat: *g*, Outer glume; *l*, lemma; *m*, palet; *p*, pericarp; *t*, testa. × 68. Stained with Flemming's triple stain. B, Cross section of a spikelet in which both kernels are badly infected: *p*, Pericarp; *s*, seed. × 85. Stained with Delafield's haematoxylin and erythrosin. C, Cross section of an immature kernel showing the relation of the various structures at that stage of development. The cells of the ovary wall (*p*, pericarp) are degenerating, and the developing seed (*s*) has almost filled the nucellar space (*n*). × 277

in the growth of the kernel. (Figs. 10, C, and 11, C.) If entrance into this tissue is effected early, the fungus advances along the intercellular spaces or from cell to cell without resistance, permeating the entire kernel (fig. 11, B). Later, the pericarp is less easily penetrated and is not so well supplied with nutrient substances as at flowering time (fig. 11, A). As the kernel matures, the layers of the testa also become a barrier to the fungus. (Figs. 10, C, and 12.) The layers of

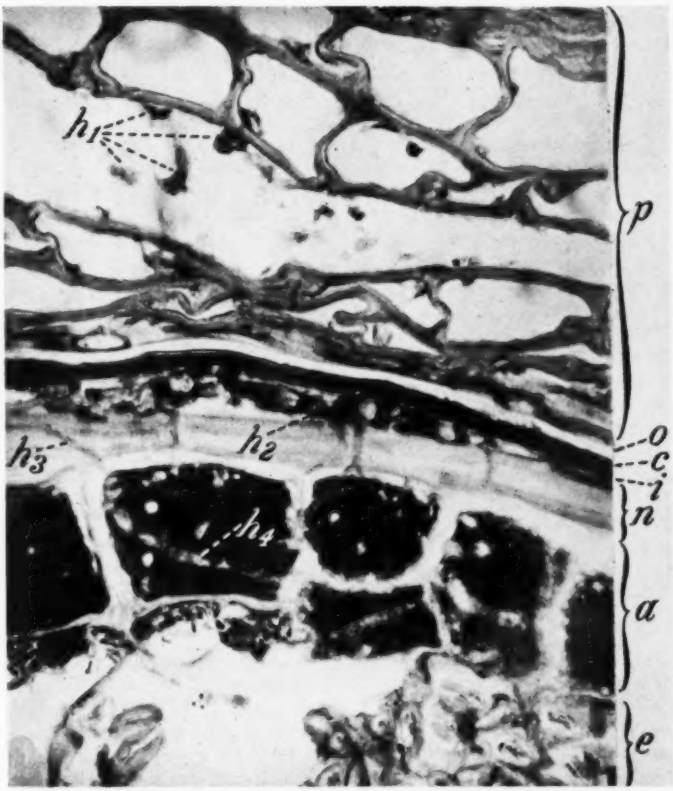

FIGURE 12.—Portion of a cross section through the outer layers of a mature wheat kernel: *p*, Pericarp; *o*, outer layer of testa (semipermeable membrane); *c*, color layer of testa; *i*, inner membrane of testa; *n*, epidermis of nucellus; *a*, aleurone cells; *e*, endosperm; h_1–h_4, hyphae. × 600. Stained with Delafield's haematoxylin and erythrosin

the testa appear to be more resistant to hyphal penetration than other parts of the kernel. In figure 12 hyphae can be seen in the pericarp (h_1), separating the inner membrane of the testa from the nucellar layer (h_2), passing through the nucellar layer (h_3), and invading the aleurone cells (h_4), but, in this portion of the section, nowhere penetrating the testa. In late infections the kernel is not permeated throughout by hyphae; whatever fungus enters the kernels is more

or less confined to the pericarp at the embryo end and to the embryo itself (*8*).

It is impossible to say how often infection originates in caught anthers, nor can it be said that infection never occurs except by the contact method with anthers or other degenerating tissues that serve as saprophytic media for early growth of the fungus. This method, however, seemed to be the rule under the conditions of the experiments described. Infection was most frequently observed to begin in degenerating anther tissue, which apparently furnishes an ideal medium for the rapid growth of *Gibberella saubinetii* under favorable conditions of temperature and moisture.

SUMMARY

Data presented in this paper are based upon macroscopic observations of inoculations with *Gibberella saubinetii*, made in the field and greenhouse, on heads of pure-line Prelude and Marquis wheat, and upon a microscopic study of serial sections of spikelets selected from these inoculation experiments.

The greenhouse inoculations were incubated in controlled-temperature and humidity chambers at 12°, 20°, 24°, 28°, and 32° C. At 28° and 32°, the relative humidity was maintained at approximately 70 to 75 per cent; at lower temperatures it was decreased to obtain the same evaporation rate from the Livingston atmometer. During the incubation period in the field, humidity was increased by bagging the heads with parchment or glassine bags.

Demineralization with hydrofluoric acid made it possible to cut serial sections of entire spikelets.

Inoculation was most effective when heads were blossoming or had just passed the blossoming stage, though infection resulted from application of inoculum from the in-the-boot stage to the past-flowering stage.

The length of the incubation period varied with the stage of development of the head at the time of inoculation and with the temperature during the period of incubation.

When heads were inoculated before blossoming, the position on the head of the first spikelet to show infection and the progress of infection to other spikelets were suggestive of the place of beginning and progress of anthesis rather than of an independent advance through the rachis. It is thought that infection occurs at flowering time, or shortly after, rather than earlier, because of the presence of anthers or other degenerating tissues in the spikelets on which the fungus first develops as a saprophyte and from which it spreads to the interior of the flower, especially to the developing kernel. Infection was frequently observed to begin in anthers that had failed to clear the glumes. Retention of anthers was not dependent on infection, for counts of healthy and infected heads of Marquis wheat showed the percentage of caught anthers to be higher for the healthy than for the infected heads. The high percentage of caught anthers occurred in the spikelet range in which initial infections were found.

Neither macroscopic observations nor histological studies indicated that initial infection occurred on the outer surface of the glumes.

Clear cases of initial infection occurring on caught anthers were found in the serial sections. The path of the fungus through the various parts of the spikelet is indicated.

It is impossible to say how often infection originates in caught anthers, nor can it be said that infection never occurs except by contact with anthers or other degenerating tissues that serve as a base of operations for the fungus. This method, however, appeared to be the rule under the conditions of the experiments described.

LITERATURE CITED

(1) ADAMS, J. F.
1921. OBSERVATIONS ON WHEAT SCAB IN PENNSYLVANIA AND ITS PATHOLOGICAL HISTORY. Phytopathology 11:115–124, illus.

(2) ARTHUR, J. C.
1891. WHEAT SCAB. Ind. Agr. Expt. Sta. Bul. 36:129–132.

(3) ATANASOFF, D.
1920. FUSARIUM-BLIGHT (SCAB) OF WHEAT AND OTHER CEREALS. Jour. Agr. Research 20:1–32, illus.

(4) ———
1923. FUSARIUM BLIGHT OF THE CEREAL CROPS. Meded. Landbouwhoogesch. [Wageningen] deel 27, No. 4, 132 p., illus. [In English. Dutch summary p. [123]–132.]

(5) LEIGHTY, C. E., and SANDO, W. J.
1924. THE BLOOMING OF WHEAT FLOWERS. Jour. Agr. Research 27:231–244, illus.

(6) PALCHEVSKY, N. A.
1891. [DISEASES OF CEREAL CROPS IN SOUTH USSURIA]. II. RESULTS OF THE INVESTIGATIONS CARRIED OUT BY M. S. WORONIN IN 1889. Translated from Russian by I. V. Krassovsky. 43 p., illus. St. Petersburg.

(7) PERCIVAL, J.
1921. THE WHEAT PLANT: A MONOGRAPH. 463 p., illus. London.

(8) PUGH, G. W., JOHANN, H., and DICKSON, J. G.
1932. THE RELATION OF THE SEMIPERMEABLE MEMBRANES OF THE WHEAT KERNEL TO INFECTION BY GIBBERELLA SAUBINETII. Jour. Agr. Research 45:609–626, illus.

(9) SZOMBATHY, K.
1918. NEUE METHODE ZUM AUFKLEBEN VON PARAFFINSCHNITTEN. Ztschr. Wiss. Mikros. u. Mikros. Tech. 34:334–336. [Reviewed by E. F. Artschwager in Bot. Gaz. 67:373–374. 1919.]

OPHIOBOLUS ORYZINUS, THE CAUSE OF A RICE DISEASE IN ARKANSAS[1]

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INTRODUCTION

In 1924, Melchers[3] quoted a report of an unidentified species of Ophiobolus on rice from Arkansas. In the fall of 1928, when rice-disease investigations in Arkansas were taken up by the writer, it was learned that the report had been made by H. R. Rosen, of the Department of Plant Pathology of the University of Arkansas, who had found the fungus on specimens of diseased rice sent in from the rice section of eastern Arkansas. It was found also that Rosen had made measurements of the asci and ascospores and had compared them with those of other graminicolous species. He had also secured cotype material of *Ophiobolus oryzinus* Sacc. from F. J. Seaver, of the New York Botanical Garden. The species of the Arkansas fungus had not been determined, however, and no inoculations had been made to establish its pathogenicity on rice plants.

Since 1928, this Ophiobolus has been found repeatedly on rice in the vicinity of Stuttgart, Ark.

The present paper reports the following steps in the investigation: (1) The description and identification of the fungus, (2) the description of the disease it produces, and (3) the results of inoculations on rice plants.

REVIEW OF LITERATURE

Three species of Ophiobolus have been reported by Miyake (*9*),[4] Ito and Kuribayashi (*6*), and Saccardo (*11*) as parasitic on rice. In addition, one other species has been reported by Baker (*1, p. 75–76*) as causing a rot of rice straw. Baker states:

> We have been making a good many observations on the rapidity of rotting of the rice stubble left in the fields. The common practice here, in harvesting, is to cut the panicles, leaving the bulk of the straw in the field, and this being difficult to handle with small native plows, is usually burned, on the ground. This is, of course, a destructive practice. Under favorable weather conditions the rotting of straw proceeds rapidly, and it soon reaches a condition which favors handling by small plows. In this process a number of very interesting fungi are concerned. * * * Three others have been proven to be new to science, *Ophiobolus oryzinus* Sacc., * * *.

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[3] MELCHERS, L. E. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1924. U.S. Dept. Agr., Bur. Plant Indus. Plant Disease Survey Bul. Sup. 40: 107–188. 1924. [Mimeographed.]

[4] Reference is made by number (italic) to Literature Cited, p. 805.

Another species, called by Hara (*5*) *Ophiochaeta graminis* (Sacc.) Hara has been reported on rice. Hara identified this fungus as *Ophiobolus graminis* Sacc., but put it into the new genus Ophiochaeta because of the presence of bristly hairs on the beak of the perithecium.

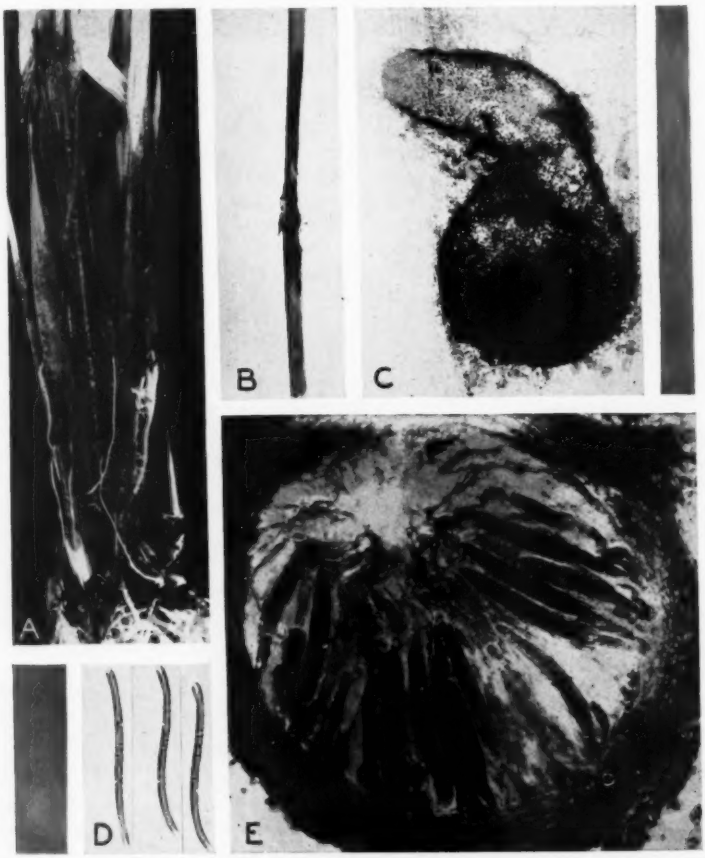

FIGURE 1.—A, Blue Rose rice plant infected with *Ophiobolus oryzinus* from inoculations made in the greenhouse at time of heading. Natural size. B, Seedling Blue Rose rice plant inoculated with *O. oryzinus* in the greenhouse. × ⅔. C, Perithecium of *O. oryzinus* partly embedded in the host tissue. × 80. D, Ascospores of *O. oryzinus*. × 300. E, Cross section of perithecium of *O. oryzinus*. × 300

THE FUNGUS

MORPHOLOGIC CHARACTERS

The Arkansas fungus may be described as follows:

Perithecia gregarious, globose to subglobose, embedded, grayish at first, becoming black to blackish brown at maturity, 187μ to 375μ in diameter; beak erumpent, hairy in culture only, 125μ to 375μ long; asci colorless, clavate or subcylindric, tapering at the ends with the apex slightly larger than the base, elongate, 8 spored, 12.5–14.5μ by 96–111μ; paraphyses present, hyaline; spores fasciculate, long and slender, 3 to 5 septate, often multigutulate, usually crescent

shaped and often slightly spirally twisted, slightly constricted at the middle septum, apex rounded, base tapering, 2–3μ by 79–112μ (mostly 86–111μ), buff to white in mass to the naked eye, but colorless when observed microscopically. On sheaths and culms of *Oryza sativa* L. (Fig. 1.)

IDENTIFICATION

A comparative study has been made of the morphologic characters of species of the genus Ophiobolus reported as causing diseases of rice, or species that might be present where rice is grown, in order to determine whether the fungus under consideration is identical with one of those previously described on *Oryza sativa*. The measurements, given in Table 1, show that several of the species differ distinctly from the fungus under investigation. In *Ophiobolus miyabeanus* Ito and Kuribayashi, *O. herpotrichus* (Fr.) Sacc., and *O. oryzae* Miyake the asci and spores are distinctly larger than in the fungus under study. However, in the other species studied, namely, *O. cariceti* (Berk. and Br.) Sacc., *O. graminis*, *O. oryzinus* Sacc., and *Ophiochaeta graminis* (Sacc.) Hara, either the asci or the spores have some characteristics similar to those of the fungus under discussion.

Comparisons were made with the specimens of *Ophiobolus graminis* on wheat obtained from Hurley Fellows and with specimens from the herbarium of the Department of Plant Pathology, University of Arkansas. Examination was made of cotype material of *O. oryzinus* collected by C. F. Baker and named by Saccardo (*12*). Two specimens of *O. oryzinus* have been examined, one from the New York Botanical Garden and one from the herbarium of the Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture. This latter specimen is No. 265, Fungi Malayana. Unfortunately, these two specimens are not identical. However, the specimen received from the Division of Mycology and Disease Survey agrees with the description given by Saccardo, while the other does not. The specimen from the New York Botanical Garden was compared with descriptions of various species of Ophiobolus known to occur on rice; it was found to agree most closely with *O. oryzae* (*9*). (Table 1.) A more detailed study, made on additional specimens, showed that this fungus was identical with specimens of *O. oryzae* found on Rexoro rice stubble in the vicinity of Crowley, La., during the winter of 1931–32.

After an examination of the available material and a study of the description of the species listed above, it was concluded that only *Ophiobolus oryzinus* agreed closely enough with the fungus under consideration to warrant further detailed comparison.

Further study of the cotype material of *Ophiobolus oryzinus* in the United States Department of Agriculture and a comparison of this material with the fungus obtained from rice in Arkansas proved that the two are at least morphologically identical. The wall structure of the perithecia was found to be the same, paraphyses were present in both specimens, and when mounted side by side the specimens were indistinguishable in color, size, and shape of asci as well as in septation, size, shape, and arrangement of the ascospores. The fungus under study was accordingly identified as *O. oryzinus*.

TABLE 1.—*Sizes of perithecia, asci, and ascospores of known species of fungi as compared with those of the species of Ophiobolus found on rice in Arkansas*

Fungus	Investigators	Host	Size of—		
			Perithecia	Asci	Ascospores
			μ	μ	μ
Sphaeria cariceti	Berkeley and Broome (*2*)	*Aira caespitosa*			76.2–101.6.
Ophiobolus cariceti	Fitzpatrick et al. (*4*)	Wheat, barley, rye, etc	330–500 (average 425)	90–115 × 10–13	60–90 (chiefly 70–80) × 3.
Do	Kirby (*8*)	Grasses and cereals			60–107 × 3–3.75.
Do	Rosen and Elliott (*10*)	Wheat		75–105 × 10.5–13.5	60–90 × 3–3.5.
Do	do	Chaetochloa		80–100 × 10.2–13	61–87 × 3–3.5.
Do	do	Festuca		85–100 × 10–13	61–88 × 3–3.5.
O. graminis	Saccardo (*11, p. 349*)	Cynodontis	500–750	80–90 × 12–13	70–75 × 3.
Do	Davis (*3*)	Wheat	300 (average)		
Do	Jones (*7*)	Oats			70–80 × 3–5.
Ophiochaeta graminis	Hara (*5*)	Rice	240–480	80–120 × 8.5–16	72.1–104 × 3.2–4.
Ophiobolus oryzae	Miyake (*9*)	do	250	125–150 × 8–10	100–130 × 2–3.
O. miyabeanus	Ito and Kuribayashi (*6*)	do	560–950 × 368–777	142–235 × 21–36	250–468 × 6–9.
O. herpotrichus	Saccardo (*11, p. 352*)	Grasses	500–750	150–185 × 9–10	135–150 × 2–2.5.
O. oryzinus[a]	Baker (*1*)	Rice	300–350	95–110 × 7–11	86–100 × 2–3.
O. sp	Tullis	do	187.5–375	96.25–111 × 12.5–14	79–112 × 2–3.

[a] Comparison made on cotype material.

CULTURAL CHARACTERS

Ophiobolus oryzinus in culture grows readily on potato-maltose, potato-dextrose and corn-meal agar, and on other solid media. Abundant felty light-colored aerial mycelium is produced on potato-maltose and potato-dextrose agar, but on other solid media the growth is less abundant. On corn-meal agar only scanty aerial mycelium is produced. On nearly all media used, the submerged mycelium became dark in the course of several days. On liquid media the fungus grew poorly and did not fruit.

With corn-meal agar perithecia are produced in abundance, either on the surface or submerged in the medium. Most of the perithecia produced on agar have rather long beaks, although the beaks of some are short.

The use of single-spore cultures as detailed below indicates that the fungus is homothallic. Forty-one single-spore isolations were made, and 37 of the resulting cultures, incubated at room temperature, produced perithecia in two weeks. In these cultures mature ascospores were produced in 17 days. This indicates that the formation of perithecia does not depend on the presence of mycelium from more than a single ascospore. The exact length of time required for spore production on the host has not yet been ascertained, but it has been found to be considerably longer than 17 days.

If the perithecia are allowed to dry slightly, the ascospores are ejected forcibly. The ascospores germinate readily if taken just after they are discharged, before they have become dry. Germ tubes are produced from both polar cells but not from the intercalary cells.

SYMPTOMS OF THE DISEASE

In several places in the rice-growing section of eastern Arkansas the disease has been observed in the field at various times since 1923, when it was first reported. Usually it is found rather late in the season, about the time when the water is drained from the fields preceding harvest. Some rotting of the sheath tissues occurs, however, before this time. Plants infected with *Ophiobolus oryzinus* are characterized by a brown discoloration of the sheaths from the crown of the plant to considerably above the water line. (Fig. 1, A, B.) In these discolored tissues the perithecia with their protruding beaks are found. The number of perithecia per plant may vary from 4 or 5 to several hundred. In the earlier stages of infection dark reddish-brown mycelial mats as much as 1.5 cm long and as wide as the sheath may be found on the inner surface of the diseased sheaths. As the lesions become older the mycelial mats increase in length, and when the sheaths become heavily infected the respective leaf blades die. At maturity the straw has a dull brownish cast very similar to that of plants attacked by the stem-rot fungus *Sclerotium oryzae* Catt. At times the two diseases have been found on the same plants or in the same fields, but parallel inoculations with the two fungi have shown that they are distinct.

Invasion of the rice culms proper by *Ophiobolus oryzinus* occurs, but perithecia are not commonly produced on them under field conditions. Under greenhouse conditions, however, perithecia have been produced on the culms.

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Ophiobolus oryzinus injures the rice plant by killing the leaves and thus reducing the photosynthetic area. In some varieties, most noticeably in red rice, the tissues at the crown also may be killed. This is less evident in Blue Rose. Furthermore, the attacked mature plants grown in the greenhouse usually produce only a single head per plant and ripen prematurely. In the field also, tillering is reduced in infected plants, and frequently only one head per plant is produced.

INOCULATION EXPERIMENTS

PATHOGENICITY OF THE FUNGUS

To determine whether the fungus was pathogenic, the following artificial inoculations were made in the greenhouse on plants of Supreme Blue Rose, Fortuna, and red rice.

On January 1, 1929, five jars of Supreme Blue Rose rice plants were inoculated at time of heading with ascospores from pure cultures of the fungus. Of the 18 plants inoculated, 5 plants in four of the jars became infected.

On February 3, 1929, another series of inoculations was made, as in the preceding experiment, on nine Supreme Blue Rose rice plants at heading time. Five plants became infected.

On January 28, 1930, 73 Blue Rose rice plants grown in 15 jars seeded September 30, 1929, were inoculated with mycelium from a single spore isolation of *Ophiobolus oryzinus* made during the summer of 1929. Sixty-four of these plants became infected.

On January 30, 1930, three jars each of Blue Rose and Fortuna rice were planted. On February 10, 30 plants in each of two jars of each variety were inoculated with a spore suspension of *Ophiobolus oryzinus*. When the readings were made on May 27, 31.7 per cent of the Blue Rose rice plants and 25 per cent of the Fortuna rice plants were infected. All the control plants of Blue Rose were free from the disease, but three of the Fortuna controls were diseased. These were probably infected by water accidentally splashed into the jar from one of the inoculated lots of plants.

Eleven seedling plants grown on corn-meal agar in test tubes were inoculated with the organism on January 20, 1930. At the end of one month the plants were dead, whereas control plants remained green and healthy. Perithecia were produced during the latter half of March on the dead seedlings.

On March 13, 1930, three jars of seedlings of red rice were inoculated with a spore suspension of *Ophiobolus oryzinus*, immediately upon emergence. Three weeks later an examination of these plants was made and it was found that an average of 36.3 per cent of the plants were infected by the fungus.

The data given in the above text show that infection of Blue Rose and Fortuna rice plants, in both seedling and heading stages, was secured by artificial inoculation with ascospores of *Ophiobolus oryzinus*. Seedlings of red rice also became infected following similar inoculation.

PATHOLOGIC HISTOLOGY

The invasion of the host is brought about by direct penetration of the outer epidermis of the two basal leaves or of the outer epidermis of the sheaths of older leaves. The two basal leaves may be invaded

directly by the hyphae. It appears, however, that appressoria are necessary for the invasion of older leaves or at least facilitate it.

Seedling rice plants were inoculated without wounding, by placing pieces of agar containing mycelium of the fungus in contact with healthy plants at the soil line. Leaves of plants so inoculated were removed and examined the second day following. The leaves were mordanted in 4 per cent iron alum, washed in water, stained for one minute in Heidenhain's haematoxylin, washed, cleared, and mounted in balsam for examination. Other material was fixed in chromo-acetic fixative, embedded, sectioned, and stained. Examination of all this material showed that the invasion was limited at first to the outer sheath, the mycelium soon penetrating to the space between the outer sheath and the one next under it. Here a mycelial mat was formed consisting of dark-colored rather coarse hyphae. Meanwhile branches of the invading hyphae penetrated the air chambers of the sheath and here balls of hyphae were produced, which developed into the perithecia. This invasion was accompanied by a slight darkening of the host tissue. Perithecia were produced for some time, and the lesion gradually increased in size until it involved the entire sheath for a distance of 4 to 6 cm from the original point of infection. Invasion of the underlying sheaths and the culm was facilitated by the production of appressoria. The tissues invaded died soon after discoloration occurred.

SUMMARY

Ophiobolus oryzinus Sacc., originally discovered by C. F. Baker on rotting rice straw in the Philippines, has been found to produce a disease of rice in Arkansas.

Inoculation experiments have shown that *Ophiobolus oryzinus* is pathogenic on Fortuna and Blue Rose rice plants in seedling and in heading stages, and on red rice plants in the seedling stage. Some of the plants have been killed outright and others have been injured through loss of leaf area. Red rice plants have been found also to have their crowns invaded. Infected plants failed to tiller normally, producing tillers only after the heading of the first culm.

Invasion of the host occurs by direct mycelial penetration of the epidermis of the basal leaves. Appressoria are formed and aid in the invasion of tissues under the basal leaves.

LITERATURE CITED

(1) Baker, C. F.
1916. Additional notes on Philippine plant diseases. Philippine Agr. and Forester 5:73–78.

(2) Berkeley, M. J., and Broome, C. E.
1861. Notices of British fungi. Ann. and Mag. Nat. Hist. (3) 7:449–456, illus.

(3) Davis, R. J.
1925. Studies on Ophiobolus graminis Sacc. and the take-all disease of wheat. Jour. Agr. Research 31:801–826, illus.

(4) Fitzpatrick, H. M., Thomas, H. E., and Kirby, R. S.
1922. The Ophiobolus causing take-all of wheat. Mycologia 14:30–37, illus.

(5) Hara, K.
1916. On Ophiochaeta graminis (Sacc.) Hara. Byôchû-Gai Zasshi (Jour. Plant Protect.) [Tokyo] 3:4–7. (342–345).

(6) Ito, S., and Kuribayashi, K.
1927. production of the ascigerous stage in culture of helminthosporium oryzae. Ann. Phytopath. Soc. Japan 2:1–8, illus.
(7) Jones, S. G.
1926. the development of the perithecium of ophiobolus graminis, sacc. Ann. Bot. [London] 40:[607]–629, illus.
(8) Kirby, R. S.
1925. the take-all disease of cereals and grasses caused by ophiobolus cariceti (berkeley and broome) saccardo. N. Y. Cornell Agr. Expt. Sta. Mem. 88, 45 p., illus.
(9) Miyake, I.
1910. studien über die pilze der reispflanze in japan. Jour. Col. Agr., Imp. Univ. Tokyo 2:[237]–276, illus.
(10) Rosen, H. R., and Elliott, J. A.
1923. pathogenicity of ophiobolus cariceti in its relationship to weakened plants. Jour. Agr. Research 25:351–358, illus.
(11) Saccardo, P. A.
1883. sylloge fungorum. v. 2. Patavii.
(12) ———
1916. notae mycologicae. Nuovo Gior. Bot. Ital. (n. s.) 23:185–234.

SCLEROTIA-FORMING HABITS OF THE COTTON ROOT-ROT FUNGUS IN TEXAS BLACK-LAND SOILS[1]

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INTRODUCTION

The cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Dug., apparently has little difficulty in living through the winters in most districts. The disease has not, however, been observed north of the southern tier of counties in Oklahoma; its present northern limit coincides approximately with the location of the Red Valley.[2] In individual areas, infection may fail to appear in successive years except in a few centers representing only a very small fraction of the original area involved. This, however, applies only to individual areas of infection and is independent of the general or regional behavior of infection.

Experiments with clean fallows have thrown much light on the manner in which the fungus perpetuates itself in the soil and lives through the years to attack the next susceptible crop planted. Plots kept in clean fallow for three or four years and then returned to cotton show a material loss in plants, indicating definitely that the fungus may remain in the soil in a viable condition for a period of several years without the presence of living host material. It is now known that sclerotia play an important part in carrying infection over into succeeding seasons.

The present paper reports the results of careful examination of the soil surrounding the first plants to die on plots that had been returned to cotton after having been in clean fallow or planted to nonsusceptible crops for several years. From extensive field examinations made during the summer and fall of 1931, it appears that the prolonged persistence of the disease in clean fallows and in plots planted to nonsusceptible crops is undoubtedly due to sclerotia formed below the plow slice and associated with various sorts of dead and decaying material such as tree stumps or old roots.

HISTORICAL REVIEW

Some of the earliest investigators (*1, 7*)[3] of cotton root rot advocated aeration of the soil as a means of control. This remedy was suggested on the assumption that the root-rot organism is a parasitic fungus living and reproducing entirely by vegetative growth. Since then, however, much additional work has been done on the fungus in culture, and many kinds of control methods have been tried in the field.

[1] Received for publication Oct. 29, 1932; issued June, 1933.
[2] Information furnished by F. M. Rolfs, of the Oklahoma Agricultural and Mechanical College.
[3] Reference is made by number (italic) to Literature Cited, p. 819.

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None of the field experiments has given consistently favorable results, which indicates that more subtle factors are involved than had been recognized.

On the assumption that the causal organism is an obligate parasite, control experiments with clean fallows were begun in 1922 and have continued since that time (*3*). Although the early results appeared promising, the short-time fallows were found to be unsatisfactory in many respects. The longer the period of clean fallow, as a rule, the greater appeared to be the control of the disease when the plot was returned to cotton. Taubenhaus and Killough (*8*) in 1923 recommended clean culture and rotation with clean culture, combined with the destruction of all winter carriers, as a means of control. In general, the results with clean culture alone have not been satisfactory, and frequently plots cropped continuously to cotton may, when the infested area breaks up, show as little loss from the disease or even less than plots that had been in clean fallow two or three years (*4*).

In 1929, Ratliffe (*6*) described a prolonged saprophytic stage of the organism, and King and Loomis (*2*) announced the discovery of a sclerotium stage of the fungus in laboratory cultures in Arizona. Later in the same year the occurrence of sclerotia at Greenville and San Antonio, Tex., was reported by Neal (*5*). With these discoveries the whole line of attack upon the problem was changed. The discovery of the sclerotium stage explained many of the inconsistencies in the behavior of clean fallow and rotation experiments. These discoveries also advanced field investigation to a stage where it was clear that the problem involved more than the control of a strictly parasitic fungus.

METHODS OF FINDING SCLEROTIA IN BLACK-LAND SOIL

The soil of the United States Cotton Breeding Field Station near Greenville, Tex., is composed largely of Wilson clay, which, even under very dry conditions, is easily broken apart in the hands, exposing the rootlets and fungus growths of current or former seasons. These growths find ready access through the soil along the well-formed cleavage planes, which are very well marked and are probably the result of years or centuries of continued movement of relatively small soil masses. Probably because the soil alternately expands when moist and contracts when dry, the effect of these irregular cleavage planes is to allow it to be readily broken apart. The surface of the cleavage planes from the lower depths is usually well coated with colloidal matter. Sometimes they have the appearance of small slickensides. These physical characteristics are very similar in the Wilson and Houston clays. (Fig. 1.)

For the first 2 to 4 inches the surface soil is loose and granular. Below this layer of loose soil is a very tight, close-grained stratum which might be termed the plowsole. Whether or not this truly represents a plowsole is a question, but such a condition was present in all Wilson and Houston clays examined. This close-grained layer of soil is usually stratified horizontally, and it is of course dissected vertically by cracks. Occasionally the fracture is conchoidal. This layer is difficult for the plant roots to penetrate, and many of the rootlets are found along the horizontal cleavage planes which separate one close-grained stratum from another. In this zone there is much less evidence of an accumulation of colloidal material along the cleavage lines than exists at lower depths.

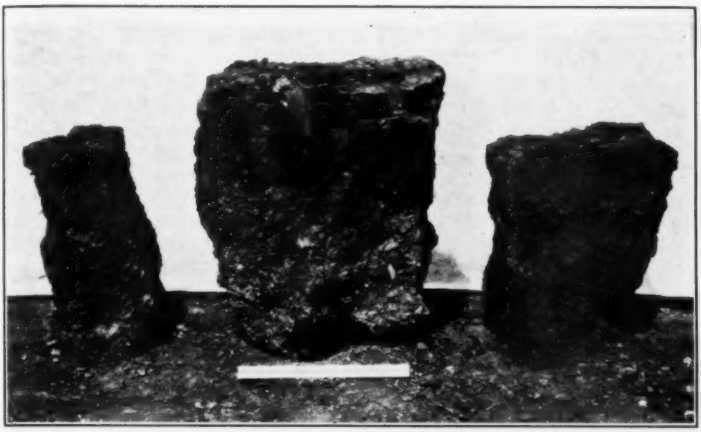

FIGURE 1.—Typical profile of Wilson clay. Note fine-grained stratum near top of the blocks. Sclerotia occur in greatest abundance below the fine-grained stratum, which is not penetrated in ordinary tillage operations. Root channels from plants grown at least five years previously are shown on the right. The loose topsoil has been removed

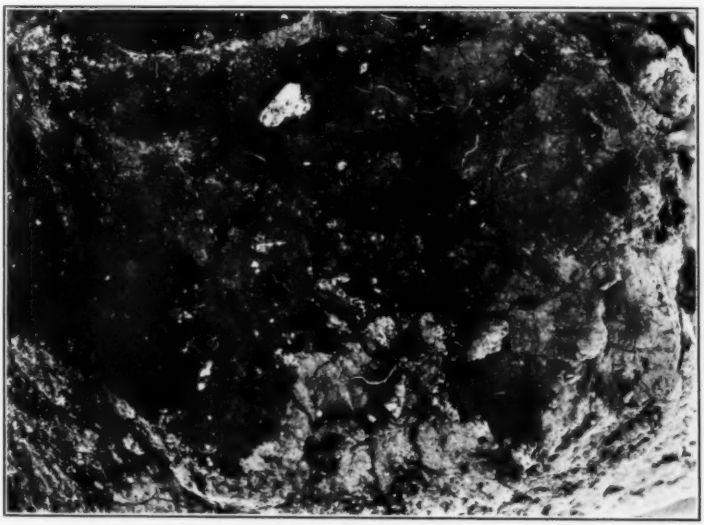

FIGURE 2.—Large clod of Houston black clay soil, outlined in black, showing natural position of viable sclerotia and cleavage planes. (Two-thirds natural size.) Photographed by P. R. Dawson. Austin, Tex., November 7, 1931

Below the plowsole, even during the driest part of the summer, the larger "chunks" can be easily broken apart with the hands and examined in the field for root-rot infection, sclerotia, and root development. Figure 1 shows the close-grained nature of the plowsole as compared with the more friable subsoil, which may frequently change abruptly in color and composition. Old root channels from a cotton crop grown five years previously are also to be seen in this figure. In these examinations sclerotia were found only in soil with a physical structure that would readily allow the penetration of rootlets and root-rot mycelium. In practically no case were sclerotia found inside a piece of compact soil so hard and tough that it could not be broken apart in the hands.

Sclerotia are readily visible in both the Wilson and the Houston black clay soils. (Fig. 2.) In soils of a lighter color and with a higher content of sand, field examinations for sclerotia are much more difficult.

The simple method of breaking the soil apart in the hands and exposing the fungus growth and the sclerotia has been found much more satisfactory than washing them out of heavy clay soils like the Houston and Wilson. This method also has the advantage of being rapid and of requiring the handling of a minimum amount of soil. It also affords a readier observation of the sclerotia in situ and makes it possible to obtain more definite information on the habits of the fungus.

OCCURRENCE OF SCLEROTIA BELOW PLOW DEPTH

The examinations were begun on July 16, 1931, and continued at short intervals for the remainder of the year. Observations were made throughout the summer and fall as often as possible, sometimes daily, but at least once every two weeks. The detailed examinations involved the handling of many tons of earth and revealed the fact that sclerotia generally are formed in groups or colonies, the great majority of which occur below the plow slice at depths usually ranging from 6 to 12 inches below the surface. A reason for the occurrence of sclerotia in greater abundance within the surface foot of soil at Greenville is that the plant roots are very sparse at lower depths. Sclerotia are evidently laid down in the soil near the small rootlets and not on the taproot or larger laterals. They are found along the old channels of the rootlets or near the fungus strands which had destroyed the small rootlets. In several cases the sclerotia were laid down in the old root channels of the 1926 cotton crop. Strands of the fungus are usually found with each colony of sclerotia. Unfortunately there is as yet no way of determining the age of sclerotia in the soil, although there is much evidence to indicate that they may be sealed in the clay and remain in a viable condition for long periods, probably several years.

INVESTIGATIONS AT GREENVILLE

SCLEROTIA IN PLOT D–1

In 1931 the west half of plot D–1 was planted to cotton, after four years of clean fallow (from 1927 to 1930), and the east half of the plot was clean fallowed for the fifth consecutive year.

Several neighboring plants died on this plot between July 20 and August 6, when the first examination was made. Diggings around the first plant that died did not yield sclerotia, but showed numerous heavy strands of hypha. Near an adjacent plant, which had been wilted only a few days, sclerotia and hyphal strands were found in abundance. Both freshly formed white sclerotia and older dark-brown ones were present. Masses of sclerotial casts were also found, indicating that sclerotia had been formed some time previously, possibly several years before, and that the growth from these old casts may have been the source of the infection present in this particular location. Most of these sclerotia were laid down near the taproot and laterals at depths of 6 to 8 inches below the surface. At 11 to 12 inches the subsoil changed from a heavy black clay to a yellowish gray clay with numerous calcareous concretions.

The third plant to die was growing in a very tenacious black clay. The surface moisture was abundant, and many shallow lateral rootlets had developed near the surface. Here, both strands and sclerotia were found within 2 inches of the top of the soil. The taproot was infected to a depth of about 10 inches, but was sound below. On an adjacent plant sclerotia were not found near the surface but at a depth of 6 to 8 inches.

A further examination of this area of infection was made on August 17. Between the first two rows next to the 5-year fallow a large colony of sclerotia was found, all of which were old and very dark brown in color. About 12 inches from a dead plant in the outside row a second colony was found which showed a large number of light-colored sclerotia, evidently very recently formed. Older sclerotia of course were found in this location also, and numerous hyphal strands in both colonies.

Another primary center of infection on this plot appeared during the last week in August. This center was represented by two dead plants. On September 28 the soil around both plants was examined and two colonies of large sclerotia were found. The first group of sclerotia was uncovered at a depth of about 5 inches below the surface and about 9 inches from the first dead plant. The second colony was found at about the same depth and about 10 inches from the second dead plant. The sclerotia in one of these groups were the largest found during the season. All appeared to be very old, but some were viable.

Another examination was made in this area on October 1, and large numbers of sclerotia and sclerotial casts were found. Most of them were found about 18 inches from one of the dead plants and at a depth of 4 to 6 inches. Since no cotton was grown on this plot in 1930, the casts probably represent sclerotia that were laid down in 1926 or 1927 and that were doubtless the primary source of infection for this center.

On September 2 a third primary center of infection was observed on the west half of plot D–1. There were seven dead plants in one row, and an examination of the soil revealed a large amount of rubbish such as bits of decayed wood, peach seeds, nails, glass, brickbats, charred wood, etc. A large colony of sclerotia was found about 10 inches from the first plant that died. Two days of continued digging failed to disclose any other source of infection, although the root-rot

map of the area for 1920 shows trees and grass within 10 feet of the location of the first dead plant.

Another primary center of infection in the same row as the one previously described was examined on November 12. This center consisted of three plants which died very late in the season. The plowsole was very well marked here, and at a depth of about 7 inches numerous old sclerotial casts and old sclerotia were found. Old peach seeds and well-rotted bits of cotton stalks or roots were found near the dead plants.

Aside from the presence or absence of dead tree roots, the sclerotia uncovered appeared to be very old, as indicated by their color, condition, and location in old root channels. The casts appeared to be the remains of sclerotia that had also been laid down several years previously. Some of the oldest looking sclerotia, which had been formed near root channels that were undoubtedly from the cotton crop of 1926, when placed in moist chambers put out new growth within two days.

Another center of infection in the same row was examined on November 13. A few old sclerotial casts were found, together with considerable organic matter which appeared to be the remains of a former rubbish pile, but no true sclerotia were discovered. The examination was resumed on November 16, and numerous sclerotia of considerable age were found. Although there is no method of determining the exact age of sclerotia laid down in the soil, indications of their having been freshly formed are found in their color and in the condition of the root material surrounding them. The fact that plants were dying in the same row at three different points and that peach seeds occurred at each of these points indicates that at some previous period there was a row of peach trees across the plot.

Since the largest body of infection in the cotton on the west half of plot D–1 was contiguous to a 5-year clean-fallow plot, an examination for root-rot infection was made in the fallow. As there were no plants to indicate the possible locations of infection in the soil, the examination was very tedious and lasted from August 24 to 28. Most of the work was done at a distance of 10 feet or more from the first row of cotton in the adjacent plot. No sclerotia were found at these distances, although one colony was discovered about 18 inches and another about 3 feet from a dead plant in the outside row of cotton next to the clean fallow. Sclerotial casts were found at a distance of 6 feet from the cotton. The decomposed remains of what appeared to be the root system of a shrub were uncovered at depths of 4 to 5 inches. This examination was rather thorough, for the area examined, but by no means conclusive. The simplest and most reliable method of testing the plot for infection will be to plant it in cotton. As in the case of the 4-year clean fallow, the presence of dead plants will show that the fungus has maintained itself for a period of practically five years without live host material.

SCLEROTIA IN PLOT D–2

Plot D–2 had been in clean fallow during the years 1927, 1928, and 1929. When the plot was planted in cotton in 1930, dead plants appeared at two points centrally located on it. The first plant that died on the plot in 1931, however, was near a corner where there had

been no mortality in 1930. On July 16, when the soil near this dead plant was removed, two large cotton stumps from the outside row of the 1930 crop were uncovered and were found to be supporting a heavy growth of the root-rot fungus. (Fig. 3.) There was no doubt of the contact between the live roots of the 1931 planting and the old roots of the 1930 planting. No sclerotia were found on July 16. A small amount of soil in this location was again examined on August 6, but no sclerotia were found. On August 17 two plants died, one next to the first dead plant and the other in the adjacent row on the inside of the plot.

On August 25 digging was resumed and two colonies of sclerotia were found about 5 feet from the first plant that died, one at a depth of 5 to 6 inches and the other at a depth of 14 to 18 inches, directly under the first. Some of the sclerotia appeared to be very old and

FIGURE 3.—Cotton stump from previous season's planting near primary center of infection, supporting a heavy growth of the root-rot fungus. Greenville, Tex., July 16, 1931

evidently had been formed before August 6, although none was found in the soil examined on that date. In one case there was evidence of the older sclerotia producing new ones by budding, as described by King and Loomis (*2*). Just below the plow depth an old peach-tree stump was found almost directly in contact with the first dead plant in the second row. This tree had been dead at least 13 years. (Fig. 4.) Large numbers of sclerotia were found near this stump, at distances of 12 to 18 inches. A point of interest in connection with this particular location is that while numerous sclerotia and sclerotial casts were found, none of them was in direct contact with either live or dead roots, but all were near dead roots and near the dead peach-tree stump. All sclerotia found were below the plow depth. Although no plants had died in 1930, when this area was planted in cotton after a 3-year clean fallow, much significance may be attached

to the fact that the first plants to die in 1931 were near the dead peach-tree stump, which was surrounded by several colonies of sclerotia, some of which appeared to have been in the soil for a long time.

The soil around a second primary center of infection on this plot was examined on July 16. The first plant had died on July 12. Although no dead material other than cotton stalks and roots was observed, large numbers of sclerotia were found, some of them evidently several weeks old. An interesting feature in connection with the examination of this center was the exposure of a shallow area in which the subsoil came within about 3 inches of the surface. The subsoil was mottled and yellowish gray in color, containing numerous small calcareous concretions. The surface soil of the Greenville station is classed as Wilson clay and is not calcareous.

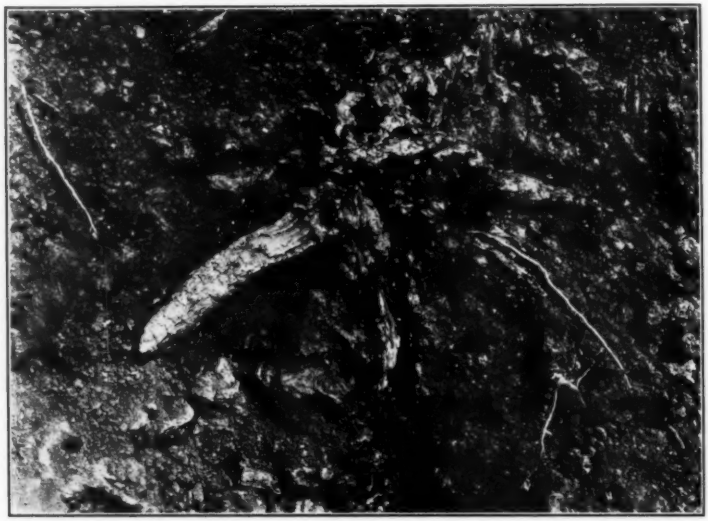

FIGURE 4.—Old peach stump uncovered below plow slice and near primary center of infection. Sclerotia were found in profusion near this stump. A history of the plot indicates that this tree has been dead for not less than 13 years. Greenville, Tex., August 25, 1931

SCLEROTIA NOT FOUND IN CONTINUOUS COTTON PLOTS SHOWING VIRULENT ROOT-ROT INFECTION

Since sclerotia had been found near all primary centers of infection on plots D–1 and D–2, which had been in fallow four and three years, respectively, it was decided to make examinations in other parts of the station.

The location selected for this study was in a field which had been continuously in cotton for several years and in which nearly all the plants had died. The area was about 900 feet distant from plots D–1 and D–2. The soil was carefully worked from around the dead plants near the central part of the infested area to several feet beyond its outer limit in the area of healthy green plants.

Trenches about 20 to 26 inches deep were dug between two rows and the soil was carefully worked down from around both the dead plants and the green plants on the outer edge of the area of infection. The trenches were usually 18 to 20 feet long, and some of them were near the center of the infested area. Examinations of this area were begun September 3 and were continued at frequent intervals until November 13; during this time many tons of earth were handled and not a single sclerotium was found. No casts of sclerotia that might have been laid down at some previous time were discovered. Several cursory examinations were made in near-by areas of infection on similar types of soil having the same crop history, but no sclerotia were found. Why this area with a heavy, early mortality of plants should have failed to yield sclerotia, when they were being found in abundance on the plots that had been in clean fallow for a period of years, is a question of genuine interest. Both shallow and deep phase soil was encountered in a number of places within the infested area, although apparently it exerted no favorable influence upon the formation of sclerotia. It is also of interest to note that repeated examinations along the outer margin of infection failed to yield sclerotia. On October 1 an examination of several primary centers of infection on plot C–6 was made, but no sclerotia or sclerotial casts were found. This plot had been in cotton continuously for at least 13 years and probably much longer.

INVESTIGATIONS IN TRAVIS COUNTY

In 1927 an experiment was begun on the farm of W. F. Voelker near Manor, Travis Co., Tex., for the purpose of determining the effectiveness as a control measure of a 3-year clean fallow as compared with that of three years of nonsusceptible crops on land very heavily infested with root rot. Two plots of approximately 1 acre each were laid off. One plot was kept in clean fallow during a 3-year period, while the other was planted to oats in 1928, sorghum in 1929, and corn in 1930.

When the plots were visited on June 23, 1931, numerous dead plants were found, probably three times as many on the clean-fallow side as on the cultivated side. When observed again, August 12, fully one-half of the plants on the clean-fallow side were dead. Upon inquiry it was found that practically all the plot that had been in clean fallow for 3 years represented the site of an old peach orchard that had died out about 10 years before. Mr. Voelker states that in 1918, when most of the orchard had died out, some of the trees were replaced with young ones that lived only a few years and died out with the older trees. This information led to the belief that if a number of the primary centers of infection were investigated the source of infection might be determined. Accordingly, on November 5 and 6 several of the primary centers were examined.

The first center of infection examined yielded a number of peach seeds, and an old dead mesquite stump about 12 inches long and about 1¼ inches in diameter. Sclerotia and sclerotial casts were found in abundance about 5 inches below the surface. (Fig. 5.)

An excavation about 6 feet square, made near the second center of infection, revealed the location of an old rubbish heap containing peach seeds, pieces of board, cans, an old broom, bones, and finally

a well-rotted peach stump. The root-rot fungus was represented by numerous large strands, but no true sclerotia were found.

The third center of infection examined revealed an old tree root, which was less than half an inch in diameter but which, when traced

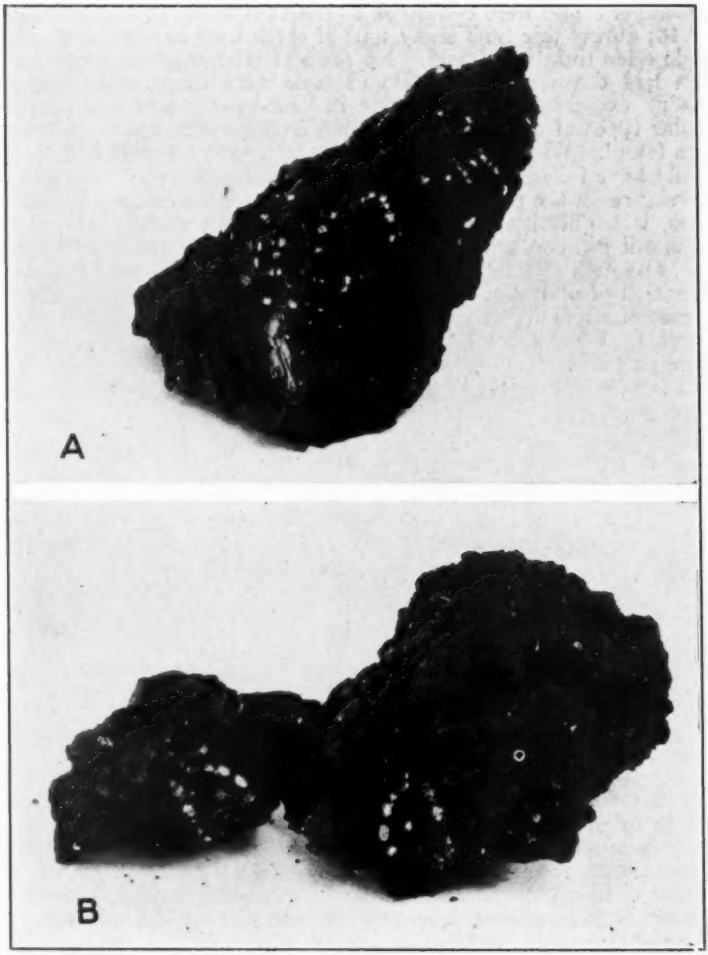

FIGURE 5.—A, Sclerotial casts as they occur naturally in chainlike formations in the soil. B, Smaller portion of clod broken away from larger portion, splitting the sclerotial casts bilaterally. (Four-fifths natural size.) Photographed by P. R. Dawson. Austin, Tex., November 7, 1931

out, was found to extend across about eight rows of cotton. Apparently the stump had been taken out and only the lateral roots left in the soil. The roots were very well rotted and numerous sclerotia and sclerotial casts were found in close proximity.

The fourth center of infection revealed the presence of an old tree root below the plowsole, which was found to connect with the old stump in the second center examined, as described above. No sclerotia were found in this center of infection.

The fifth center of infection yielded abundant sclerotia and sclerotial casts at depths of 10 to 12 inches.

Three other examinations were made in the 3-year-fallow plot, two of which yielded a number of peach seeds but no sclerotia, while one gave neither sclerotia nor other evidence of the source of the infection. These later examinations, however, were not so thorough as they might have been, and possibly the seat of infection in these points was overlooked.

Negative evidence in cases of this kind is not entirely reliable. The source of infection might be small and in many cases might have been destroyed or even overlooked unless much time were spent in the tedious process of examining the field.

A point of unusual interest was revealed in the examination of a well-defined primary center of infection on the plot that had been planted to nonsusceptible crops for three years. In examining this center, sclerotia and sclerotial casts were found in great abundance. Near one side of the area of infestation two small mesquite stumps were discovered which had been dead for years and around which was a great number of sclerotia. A rather sparse growth of mycelium extended over the mesquite stumps, and sclerotia were present 2 to 4 inches from the stumps and 6 to 15 inches below the surface. According to the owner of the land, the plot had been put into cultivation in the spring of 1884 and had had a crop planted on it every year since.

Recurrence of root rot after several seasons of clean fallows or of nonsusceptible crops is explained by deep-seated infestation of the fungus below the plowsole. The fungus was found in many cases on decayed roots of trees buried in the soil for long periods, in some instances for 30 or 40 years.

INVESTIGATIONS IN ROCKWALL COUNTY

On November 19 an examination of the soil was made around several primary centers of infection on the farm of B. F. Weatherford, near Rockwall, Rockwall Co., Tex. The soil would probably be classed as an eroded phase of Houston clay. The loss from root rot on this type of soil is extremely high.

The field examined had been planted to oats in 1928 and 1929, to millet in 1930, and to cotton in 1931. Each summer the plot was kept in fallow, and practically no weeds were allowed to grow on the area.

On July 27 there was a scattering of dead plants over the plot, some of which had died a few weeks earlier. After the mapping on November 19, an examination was made of five primary centers of infection. Four of the centers yielded sclerotia and sclerotial casts in abundance, but in one of the locations no sclerotia were found. Two explorations were made in an area of general infestation, but no sclerotia were found.

SUMMARY AND CONCLUSIONS

In the study of the sclerotia-forming habits of the root-rot fungus (*Phymatotrichum omnivorum* (Shear) Dug.) reported herein, a simple method of investigation was employed in which the soil was excavated from different depths and broken apart in the hands. Thus the sclerotia were observed in situ, and their relation to small root channels and cleavage planes of the soil was readily seen. It was difficult to wash the sclerotia out of the black clay soil, but by breaking the clay apart it was easier to find them than in the more friable soils.

To determine the seats of infection the soil was carefully excavated and examined to depths of at least 24 inches around numerous primary centers where the first plants died of root rot. These examinations were made throughout the summer and fall of 1931, in fields planted continuously to cotton, in others planted in cotton for the first time after 3-year or 4-year clean fallows, and in still others where cotton followed three years of nonsusceptible crops.

Sclerotia were frequently found in the soil, usually in colonies or groups rather than singly or sparingly.

After the seat of infection in a primary center had been located, continued digging failed to reveal other sources. Although tons of additional earth were removed, no more sclerotia were found.

Older sclerotia were found below the plow slice at depths of 4 to 18 inches, most of them occurring at a depth of 4 to 12 inches.

Sclerotia were not found on the taproots of the cotton plants or on the large lateral roots but out in the soil where only small rootlets occurred.

Newly formed sclerotia were found only during the period from August 6 to 17.

No sclerotia were found in plots planted continuously to cotton where most of the plants had died of root rot. They were, however, found in abundance in primary centers of infection in fields where cotton followed 3 or 4 years of clean fallow or 3 years of nonsusceptible crops.

Sclerotia were frequently found in large numbers near old tree roots or stumps that had been dead many years. Some of the primary centers of infection, however, although near old dead roots, failed to yield sclerotia.

Most of the primary centers, after three years of nonsusceptible crops or 3-year fallows, yielded either sclerotia or infested old decayed material of some kind, or both.

Where deep-seated infestations exist, control of root rot by clean fallows or nonsusceptible crops apparently will need to be supplemented by tillage operations that will reach the sclerotia, which are usually found below the plow slice at depths of 4 to 12 inches.

Considerable significance may be attached to the fact that in most of the primary centers examined sclerotia were abundant in plots that had been planted to nonsusceptible crops or kept in clean fallow for several years, whereas no sclerotia were found in plots planted continuously to cotton. From these facts it may be inferred that the sclerotia, which apparently are capable of long periods of quiescence, are connected primarily with the saprophytic phase of the fungus and serve as a resting stage.

LITERATURE CITED

(1) DUGGAR, B. M.
1926. THE TEXAS ROOT ROT FUNGUS AND ITS CONIDIAL STAGE. Ann. Missouri Bot. Gard. 3:11–23, illus.
(2) KING, C. J., and LOOMIS, H. F.
1929. FURTHER STUDIES OF COTTON ROOT ROT IN ARIZONA WITH A DESCRIPTION OF A SCLEROTIUM STAGE OF THE FUNGUS. Jour. Agr. Research 39:641–676, illus.
(3) MCNAMARA, H. C.
1926. BEHAVIOR OF COTTON ROOT ROT AT GREENVILLE, TEX., INCLUDING AN EXPERIMENT WITH CLEAN FALLOWS. Jour. Agr. Research 32:17–24, illus.
(4) ——— HOOTON, D. R., and PORTER, D. D.
1931. CYCLES OF GROWTH IN COTTON ROOT ROT AT GREENVILLE, TEX. U. S. Dept. Agr. Circ. 173, 18 p., illus.
(5) NEAL, D. C.
1929. THE OCCURRENCE OF VIABLE COTTON ROOT-ROT SCLEROTIA IN NATURE. Science (n. s.) 70:409–410.
(6) RATLIFFE, G. T.
1929. A PROLONGED SAPROPHYTIC STAGE OF THE COTTON ROOT-ROT FUNGUS. U. S. Dept. Agr. Circ. 67, 8 p., illus.
(7) SHEAR, L. C., and MILES, G. F.
1908. TEXAS ROOT-ROT OF COTTON: FIELD EXPERIMENTS IN 1907. U. S. Dept. Agr., Bur. Plant Indus. Circ. 9, 7 p., illus.
(8) TAUBENHAUS, J. J., and KILLOUGH, D. T.
1923. TEXAS ROOT-ROT OF COTTON AND METHODS OF ITS CONTROL. Texas Agr. Expt. Sta. Bul. 307, 98 p., illus.

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ARTIFICIAL TRANSMISSION OF SUGARCANE MOSAIC[1]

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INTRODUCTION

Sugarcane mosaic is a disease caused by a conceivable but as yet undistinguishable virus, which has been recognized during the last decade as affecting sugarcane (*Saccharum officinarum* L.) to some extent in almost every region where sugarcane is grown commercially. For a long time investigators failed in their efforts to infect plants experimentally with the virus of this mosaic disease, and the question arose as to whether it should be considered infectious or whether the symptoms observed in sugarcane might be caused by inherent degeneration or by a nonparasitic disease. The truly infectious nature of sugarcane mosaic was firmly established about 10 years ago, but many problems still remain unsolved.

Concerning the character of the virus in the extracted plant juice, the general belief has been that if manipulated in the presence of air it would wholly, or at least largely, lose its infectiousness, particularly if inoculations were not made immediately after extraction. This belief, which was not thoroughly tested, became a serious handicap in the study of the virus. This paper presents a procedure developed during the past year for the artificial transmission of the mosaic disease, which offers not only considerable freedom in the manipulation of the extracted material but also permits the use of the virus-containing extract for a considerable time after storage. The results here recorded show that exposure to the air does not necessarily impair, under all conditions, the disease-producing properties of the extract of diseased tissues. The studies were begun in the autumn of 1929 at Johns Hopkins University and have been continued in the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture.

HISTORICAL REVIEW

The disease now known as sugarcane mosaic has been the subject of investigations and reports by various workers, first in Java (*31*),[3] later in Hawaii (*25*), and more recently in the West Indies (*11*, *16*, *30*, *33*, *34*, *35*, *36*, *37*), the United States of America (*6*), Argentina (*17*), Natal (*27*), and India (*29*). In 1892 Van Musschenbroek (*31*) gave a description of the disease under the symptomatic name "gele strepenziekte," or yellow-strike disease, and in 1893 there appeared a multicolor reproduction showing a portion of a sugarcane leaf affected

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[2] Acknowledgment is made to Dr. E. W. Brandes, principal pathologist in charge, Division of Sugar Plant Investigations, for his contribution of many observations and much constructive criticism, and to Prof. Burton E. Livingston, of the Johns Hopkins University, for his help in the presentation of the results.

[3] Reference is made by number (italic) to Literature Cited, p. 837.

with the disease, together with a note on its occurrence, by Arendsen Hein (*3*). Since 1919 the disease has been recorded (*6*, *7*, *8*, *21*, *39*) as affecting not only sugarcane and other species of Saccharum but also maize (*Zea mays* L.); sorgo (*Sorghum vulgare* Pers.) and other species of Sorghum; millet (*Pennisetum glaucum* (L.) R. Br.); and the wild grasses *Digitaria sanguinalis* (L.) Scop., *Paspalum boscianum* Flügge, *Setaria lutescens* (Weigel) Hubb., *S. magna* Griseb., *Panicum dichotomiflorum* Michx., *Eleusine indica* (L.) Gaertn., *Echinochloa crusgalli* (L.) Beauv., *E. colonum* (L.) Link, and *Brachiaria extensa* Chase.

In 1903 Kamerling (*19*), working in Java, stated that he had transmitted the disease to healthy sugarcane by inoculation with juice expressed from mosaic-diseased sugarcane. His inoculations were made by injection. Although he did not specify the kind of instrument used, it was presumably a hollow-needle syringe, since he stated that he had followed the technic employed by Beijerinck (*4*) in transmitting tobacco mosaic to healthy tobacco plants, and Beijerinck stated that he used a hollow-needle syringe ("Pravaz'schen Spritze"). Kamerling, regarding this sugarcane disease as infectious, placed it in the same class with the well-known mosaic disease of tobacco. His tests, however, do not appear to have fully justified such an unqualified conclusion, for some of his uninoculated and apparently healthy plants contracted the disease in the course of his experimentation. He admitted the possibility that the disease might have been transmitted in a very slight degree by air, but he expressed the conviction that at least some of the plants which he inoculated with diseased juice contracted the disease directly because of his inoculation. On the other hand, Van der Stok (*38*) in 1907, Kobus (*20*) in 1908, and Wilbrink and Ledeboer (*42*) in 1910—all of whom, like Kamerling, studied the disease in Java—were unable to infect healthy sugarcane plants by any of the methods they tried, including the procedure described by Kamerling.

In 1917 Stevenson (*35*, *36*) reported the results of a study of sugarcane mosaic in Puerto Rico, referring to it as the "new disease," "the mottling disease," "an epiphytotic of cane disease," etc. It was not until 1919, however, that he identified the disease as it occurred in Puerto Rico with the gele strepenziekte, or yellow-stripe disease, described by others. Working thus independently he suspected the disease to be due to an infectious principle (*37*) and carried out an extensive series of inoculation tests, not only employing expressed juice from diseased cane but also inserting diseased cane tissue in incisions made in healthy plants; but he did not succeed even once in causing the disease symptoms to appear in the inoculated plants.

Stevenson's paper of 1917 (*35*) aroused the interest of other investigators, particularly H. L. Lyon (as reported by Colón (*14*)), of the Hawaiian Sugar Planters' Association, who regarded this disease as identical with the disease known as gele strepenziekte, or yellow-stripe disease, in Java, Hawaii, and elsewhere. The yellow-stripe disease had been studied by Lyon in Hawaii from 1911 to 1914 (*25*). In 1921 (*26*) he reported that in open-field experiments he had observed it in some of the plants which he had inoculated with expressed juice. Similar tests in the greenhouse failed, however, to produce the disease.

The identity of the disease causing mottling of sugarcane in Puerto Rico with the gele strepenziekte of Java and the yellow-stripe disease

of Hawaii and other regions was finally recognized by the investigators of this subject in Puerto Rico (*6, 14, 16, 30, 37*), and the infectious nature of the disease was generally accepted by them. Adequate evidence of its being infectious was still lacking, however; the experimental evidence was meager and not free from the possibility of error; and nothing was known regarding field dissemination.

In 1920 Brandes (*7*) published the outstanding discovery that sugarcane mosaic is transmitted naturally by *Aphis maidis* Fitch. Additional evidence showing the infectious nature of this mosaic and the rôle played by *Aphis maidis* in its dissemination was presented by Bruner (*11*) in Cuba, by Kunkel (*22*) in Hawaii, by Ledeboer (*24*) and Wilbrink (*40*) in Java, by Chardon and Veve (*12, 13*) in Puerto Rico, and by Fawcett (*17*) in Argentina.

Early in 1920, Brandes (*7*) succeeded in transmitting the mosaic disease directly from diseased to healthy sugarcane without the aid of *Aphis maidis*. By means of a glass hollow-needle syringe, he injected into the growing points of healthy sugarcane plants 0.5 cc of sap pressed from ground tissue of young joints of mosaic-diseased sugarcane. After an interval of a little more than one month, infection had appeared in 8 out of 10 plants inoculated with sap expressed under a cover of purified mineral oil, but only 2 out of 10 inoculated plants contracted the disease when the sap employed was obtained without any protective covering against exposure to air. It is important to note, however, that in both instances the inoculating material was used, as the author states, "immediately after being prepared." [4]

Brandes and Klaphaak (*10*) regarded lapse of time and exposure to air as the chief causes of the failure of expressed virus-containing juice to produce the disease. They say (*10, p. 251*)—

> Virus capable of causing infection when used immediately after being expressed from diseased stalks was found in one experiment to be without effect when injected 24 hours later.[5] The virus of grass mosaic is less stable or more sensitive to the influence of its environment than that of many other similar diseases, notably the tobacco mosaic. In these experiments it has been found very refractory and difficult of physical manipulation or chemical treatment without loss of virulence.

They also remark (*10, p. 248*): "Sometimes whole series of inoculations including known susceptible control plants failed, owing to unknown causes." These authors continued to use a cover of mineral oil over their inoculum, and they succeeded in transmitting the disease directly from cane to cane and from cane to sorgo, millet, and *Digitaria sanguinalis*. Thus it was definitely established not only that sugarcane mosaic is really infectious and transmissible to healthy plants of sugarcane and some related forms by the vector *Aphis maidis*, but also that it may be artificially transmitted by means of hollow-needle inoculation with freshly extracted sap from diseased plants suitably protected from the air.

Doolittle (*15*) found that juices of mosaic cucurbits retained the power of infection for only a short period after their extraction and usually lost their virulence within 24 to 48 hours. Dried material

[4] In this connection Brandes (*7, p. 132*) credits F. S. Earle with calling attention, in a then unpublished paper, to a method of inoculating with juice expressed under oil to hinder possible oxidation. Bruner (*11*), however, states it as his understanding that the procedure of extracting the cane juice under mineral oil to avoid oxidation was originally suggested by E. D. Colón and F. A. Lopez Dominguez, of the insular experiment tation of Puerto Rico, and that this procedure was employed later by Professor Earle.

[5] This was not intended to imply that the virus would lose its virulence at the end of 24 hours under all conditions, as Doctor Brandes, since his first experiments in 1920, had been storing away samples of extract of sugarcane mosaic for the purpose of testing in the future the effect of age on retention of virulence.

likewise suffered a rapid loss of the infectious power. Preservatives to stop fermentation did not serve to prolong the period of activation in extracted infected juice, and low temperature had only a slight effect in prolonging the power of infection. Henderson and Wingard (*18*) report that the tobacco ring-spot virus is inactivated in 12 to 24 hours at ordinary room temperature and that they never succeeded in getting infection from dry material, no matter how fresh it happened to be; on the other hand, they found that the virus may retain its virulence for at least 22 months when kept at a temperature of −18° C.

Allard (*1*, *2*) found that the tobacco-mosaic virus was still infectious after it had been bottled for four months. He later found (*2*, *p. 636*) that at the end of 231 days similarly kept extract of tobacco-mosaic virus was still highly infectious.

Earle (*16*, *p. 17*) states that—

> oxidation might affect the vitality of the mosaic virus, and that a sucking insect flying from a diseased to a healthy plant and again feeding might regurgitate a minute quantity of the diseased juice without having exposed it to the air.

With juice pressed from diseased canes, without oil covering, he inoculated 7 canes by means of a hypodermic needle thrust into the leaf spindle above the terminal bud. After about 6 weeks he observed 2 infected plants, whereas from 10 inoculations made with oil-protected diseased juice he observed 5 infected plants after about 4 weeks. Seven other plants, inoculated in the midribs of young leaves, showed no symptoms of the disease. On the day after the tests with oil-protected juice some of the juice was used in 3 additional cane plants, but no positive infection developed. Because of the imperfectly known technic of inoculation and the utter lack of understanding of host response and reaction, little could be said in favor of one procedure over the other, particularly when there seemed to be a lack of uniformity in the results, even with the method that had been successful in one instance.

At about the same time the writer (*30*) obtained 2 mosaic infections among 5 cane plants that had been inoculated with air-exposed infected juice. In another similar experiment he obtained 2 infections out of 20 inoculations. In both tests the period between inoculation and the appearance of the first mosaic symptoms was about 14 days. The tests of both Earle and the writer, however, unlike those of Brandes, were performed under conditions that did not preclude the possibility of natural infection by aphids.

Following the studies of Brandes, Earle, and the writer, Bruner (*11*) attempted to transmit sugarcane mosaic, sometimes through the agency of *Aphis maidis* and sometimes without employing the insect vector; he also considered the possibility that exposure to air might have an inhibiting effect upon the virus in pressed-out sugarcane sap. To reduce the air exposure of the sap intended for inoculation he superimposed a mosaic-infected leaf upon a healthy leaf and, holding the two in close contact by finger pressure, he thrust a fine hypodermic needle rapidly through the infected leaf into the healthy one. In this manner he inoculated a tender leaf, a mature leaf, and an old leaf on each of 100 shoots comprising altogether 23 cane stools of the variety Cristalina. The experiment was made November 6, 1920; and in March, 1921, only eight shoots in 3 stools showed infection. Bruner believed that the small percentage of

infection in this experiment might have been due to the fact that his inoculated plants were well advanced in development and the rate of growth was slow. In another experiment (his No. 8) Bruner injected infected juice that had been extracted under a cover of oil into 15 sugarcane plants of the variety Cavengerie, while 15 other plants of the same variety were similarly injected with juice extracted in the air. The operations were performed rapidly and in each series only one minute intervened between extraction and inoculation. In each of the lots 3 plants became infected within about 8 weeks from the time of inoculation. Bruner (*11, p. 21*) states (translation):

> We can conclude that the diseased cane juice extracted without any provision against oxidation will reproduce the mosaic just as the juice extracted under a protective cover of oil if there is no delay in making the inoculation after extracting the juice.

In 1920 Brandes (*7*) obtained no infection of cane plants of the Lahaina variety when juice from infected leaves, prepared without any oil protection, was rubbed with the fingers into either unbroken or needle-scarified surfaces of young leaves; nor did any infection result when inoculation was accomplished by scarifying the young leaf cells with a sharp needle dipped in the air-exposed mosaic-infected juice. Only 1 infection resulted from 10 inoculations made by numerous needle pricks with the same air-exposed juice. In other tests, where the inoculation methods just mentioned were employed except that the diseased juice was pressed from young stems under mineral oil, there were no infections. When a hypodermic syringe was used, 8 out of 10 inoculations made with oil-protected inoculum were successful, whereas only 2 out of 10 inoculations made with unprotected inoculum were successful. Using absorbent cotton wet with juice that had been pressed (without oil) from leaves and upper joints of diseased Lahaina sugarcane, Kunkel (*23*) rubbed the inoculum into wounded leaves of healthy Striped-Tip sugarcane plants. He performed 3 experiments, each with 6 plants, but reported no infections except from the third experiment, after a lapse of about 3 months, when 5 of the plants showed mosaic symptoms. From 2 to 3 weeks usually intervenes between the date of inoculation and the first appearance of symptoms of this disease.

McRae and Subramaniam (*29*), using a needle, pricked juice from freshly crushed mosaic leaves into the leaf sheaths and stems of sugarcane, maize, and sorghum. They reported many successful inoculations, but the proportion of infection to inoculation was highly variable; for instance, out of 16 shoots of the cane variety Coimbatore 213, inoculated with juice from diseased Red Mauritius cane, all showed the disease; but out of 30 shoots of Coimbatore 213 inoculated with juice from Coimbatore 205 only 2 developed the disease. While this variability may perhaps have been due in some degree to differences in host relationships and possible attenuation of the virus in some varieties, it seems probable that the method by which the inoculum was secured and introduced into the healthy tissue was often at fault.

Sein (*32*), using a fine needle, pricked freshly expressed diseased juice (without any oil covering) into the tender parts of the tightly rolled young leaves of cane shoots and obtained more than 50 successful infections in one experiment with 100 inoculated plants. Subsequently he abandoned the use of extracted sap and adopted the

principles of Bruner's (*11*) method; a band of expanded mosaic leaf was held tightly wrapped around the stripped cylinder of young, tender leaves of the healthy plant and the pin point was repeatedly thrust through the diseased tissue into the healthy leaves. Out of 100 shoots inoculated in the open field by this method, 94 per cent showed infection. Sein thought that his very fine needle, thrust rapidly through the previously unbroken band of diseased leaf into the still tightly rolled cylinder of healthy young leaves, carried diseased material almost directly into healthy tissue without any considerable exposure of the material to the air. It is apparent, however, that if exposure to air is really detrimental to this virus, the air between the two rather rough leaf surfaces may be sufficient to affect the virulence of the minute quantity of infected juice carried by the needle, no matter how firmly the band of diseased leaf is held by the fingers against the leaf cylinder. The advantage of using a very fine needle, as recommended by Sein, would come from the fact that the very small stab lesion resulting from the puncture would cause very little injury, and that the virus would thus be introduced directly into an environment of living cells.

A study of these recent papers on the artificial transmission of sugarcane mosaic makes it evident that their authors have generally thought that this virus (or at any rate the expressed juice from diseased tissues) becomes greatly weakened or loses its capacity for producing mosaic symptoms if the inoculum is not used very soon after it is prepared, and especially if air has access to it during preparation or in the process of inoculation.

It appears, though it has not been exactly thus stated, that the disease-producing capacity of the virus of sugarcane mosaic depends on its being in intimate and practically uninterrupted association with the living cell contents of its host. No one has as yet recorded the occurrence of infection in healthy sugarcane plants from the application of decayed or dried material from infected sugarcane plants or from air-exposed extract of diseased cane tissue when the extract has been kept a long time at ordinary room temperatures. It is commonly supposed that there are marked differences in susceptibility to visible infection, not only among different varieties of cane at any given stage of growth but also among the several parts or regions of an individual plant. The inoculum from diseased plants has generally been applied to the growing point of the cane plant or the youngest leaves, and it seems to be an accepted conclusion that cane roots are not at all receptive to the introduction of the disease. The capacity of the virus to produce the symptoms of sugarcane mosaic seems to depend on a narrow range of external conditions as well as on the varying internal reactions of the host.[6]

EXPERIMENTAL METHODS

The inoculum used in the experiments reported in this paper was obtained from young mosaic-infected suckers which sprang up from time to time from the bases of three mosaic-infected fully grown stools of the sugarcane variety P. O. J.[7] 234. After the mature leaves had been discarded the remaining leafy portions, including the false

[6] The inoculation method employed by McKinney (*28*) in his work with wheat and rye was tested, but in no instance did the inoculated sugarcane show infection.

[7] Proefstation Oost Java.

stems, of about 10 shoots were cut into ½-inch lengths and ground in a meat chopper, slowly, in order to avoid overheating the material. The sharply grooved nut-butter-cutter disk furnished with the chopper was employed. All apparatus used had been sterilized by boiling in hot water. The ground material was received from the chopper in a 20-ml porcelain evaporating dish, most of the liquid being then squeezed out by hand through cheesecloth. The nearly dry pulp was discarded and the green filtrate, which contained much material in suspension, including whole and broken chloroplasts, was stored in vials or flasks, without special precautions of any kind except, in some instances, in regard to temperature.

When a cane shoot was to be inoculated some of this liquid was transferred by means of a pipette to the wedge-shaped opening between the youngest expanded leaf blade and the next younger leaf on the same side, which was still rolled. The blade of the youngest expanded leaf bends sharply away from the vertical axis of the plant, and its base partly clasps the false stem or leaf spindle, thus forming a sort of cup or reservoir that retains the liquid, while a small area of the outer surface of the most recently exposed but still rolled leaf is covered by the liquid. The relations of these two leaves and the expressed juice held between them are shown in Figure 1. A fine needle point (usually No. 216, Special Minuten Nadeln), set into a glass rod which served as a handle, was next passed horizontally, or somewhat obliquely downward, through the liquid and into the submerged area of the still rolled leaf. In addition to the direct downward punctures, several vertical cuts with the needle were made through the leaf tissue in order to allow the contact of the virus with the now severed fine transverse connections of the vascular bundles. This stabbing operation was repeated rapidly about 5 or 6 times, with gentle pressure; each needle thrust formed a new puncture, but all punctures were close together and were covered by the juice in the reservoir. This method of inoculation was found to be suitable for young sorghum and Digitaria plants as well as for sugarcane. Inoculations were usually made on a cloudy afternoon, when the light intensity was weak and evaporation was slow.

The inoculated plants stood for many weeks in pots of soil in an ordinary greenhouse, at a temperature varying from about 25° to 30° C., and were frequently inspected. Each inoculated culture had a green label showing how and when inoculation was performed. When mosaic symptoms appeared a red label was attached, bearing the date of the first appearance of the disease. Subsequent notes were added as the malady progressed.

The greenhouse was screened, and no individuals of *Aphis maidis*, the natural transmitter of the disease, were observed in the compartments used or in adjacent ones. As has been said, no special precautions were taken to protect the expressed juice from the air. Nor were any pains taken to use the extract immediately, although many inoculations were made on the same day as that on which the extract was prepared—usually after about 2 to 4 hours. When inoculations were not to be made until the day after the extraction or later, the inoculum was stored in open beakers or vials in an electric refrigerator at a temperature of about 4° C. At this temperature, as well as at higher ones, considerable development of bacteria and other microorganisms was observed after 24 hours, and it was suspected that these

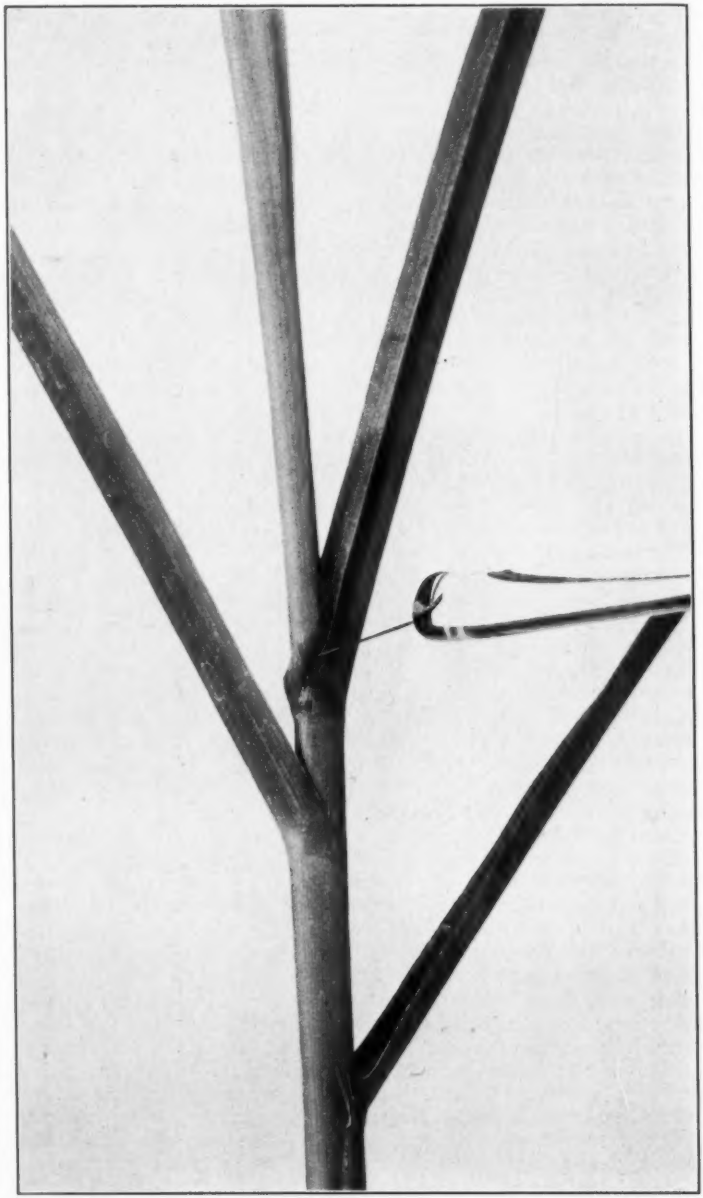

FIGURE 1.—Inoculation of a sugarcane shoot with extracted juice of a mosaic-infected plant. A drop of infected liquid is placed at the wedge-shaped opening between the youngest expanded leaf blade and the next younger, still rolled leaf. The fine needle point is passed through the liquid and into the liquid-covered area of the rolled leaf

might partly account for some inactivation of the stored extract. Tests in which stored extract was kept at a temperature below the freezing point of water were therefore made. For these tests several somewhat different procedures were tried. (1) Freshly expressed juice from infected cane was kept in open vials in the freezing compartment of an electric refrigerator from within 1 hour after the crushing of the diseased material until the inoculum was used. The temperature of this compartment was −6° C., and the liquid became solidly frozen within 24 hours, being thawed at room temperature just before use. (2) Fresh diseased extract in similar open vials was frozen solid within 20 minutes after extraction, by means of blocks of solid carbon dioxide placed alongside in a small, suitably insulated box. The vials of frozen inoculum were then transferred to the refrigerator compartment just mentioned, where the material remained frozen, at about −6° until thawed for use. (3) Fresh juice from mosaic sugarcane was first stored for 6 days at 4°; it was then transferred to the freezing compartment of the refrigerator (−6°) and stored there until thawed for use.

EXPERIMENTAL DATA

INOCULATION TESTS

Data for several hundred inoculation tests made in the spring and summer of 1931 are presented in Table 1. In all these the inoculum employed was juice expressed from mosaic-diseased sugarcane of the variety P. O. J. 234, and the method of inoculation was usually that described in the preceding section. It is, of course, understood that a single inoculation of this kind includes several separate needle punctures, all close together. This type of inoculation is herein designated as the "regular" method. In a few instances other methods of inoculation were used. Tests were made on the Black Amber variety of sorgo and on six mosaic-susceptible varieties of sugarcane, namely: Louisiana Purple, P. O. J. 36–M [8], D [9]–74, P. O. J. 234, Cristalina, and B. H. [10] 10/12. In each experiment a young, vigorously growing, and apparently healthy plant 1 to 2 feet in height was employed for each inoculation. The results of these tests are shown in Table 1.

Specific records of control plants are omitted, since such records would constitute merely a series of repetitions. In each test, however, control plants as nearly like the inoculated plants as possible were used. The controls received the same treatment as the inoculated plants except that they were not inoculated. The number of controls was usually the same as the number of inoculated plants, but in some instances it was much larger. Several hundred healthy surgarcane plants growing in the greenhouse were continually under observation until March, 1932, and these served as additional, though undesignated, controls. None of the uninoculated plants, whether designated as controls or not, showed any mosaic symptom during the period of observation (from April, 1931, to March, 1932). As the records show, however, inoculation did not always produce infection.

[8] Mingka selection of P.O.J. 36.
[9] Demerara.
[10] Barbados hybrid.

TABLE 1.—*Results of inoculating Black Amber sorgo and six susceptible varieties of sugarcane with mosaic virus under various conditions in 1932*

Variety	Experiment No.	Inoculum		Inoculations			Infections observed		
		Age	Storage temperature	Date	Number	Method	Date	Number	Total
		Hours	° *C.*						
	1	2–4	(a)	Apr. 27	12	(b)	May 11	1	8
							May 23	4	
							May 26	3	
							do	1	
	2	24	4	Apr. 28	12	(c)	May 28	2	8
							May 29	1	
Sorgo (Black Amber)							June 4	4	
	3	24	4	do	12	(d)	May 11	1	2
							June 3	1	
	4	2–4	(a)	May 27	6	(c)		0	0
	5	2–4	(a)	do	6	(c)	June 12	1	3
							June 18	2	
Sugarcane:									
							May 11	1	
	6	24	4	Apr. 28	6	(c)	May 15	1	5
							May 24	2	
							May 25	1	
							May 28	1	
	7	24	4	May 15	13	(c)	June 1	1	7
							June 8	1	
							June 17	4	
							May 28	1	
Louisiana Purple	8	24	4	do	12	(f)	June 8	1	3
							June 17	1	
	9	2–4	(a)	May 25	11	(c)	June 7	5	6
							June 9	1	
	10	2–4	(a)	do	10	(c)		0	0
		Days							
	11	11	4	do	5	(c)	June 8	1	1
	12	10	4	June 23	11	(c)	July 24	3	3
		Hours							
	13	2–4	(a)	May 27	6	(c)	June 18	4	5
							June 22	1	
		Days					July 17	1	
	14	26	4	June 19	6	(c)	July 22	1	3
							July 23	1	
							July 31	1	
							Aug. 5	5	
							Aug. 6	5	
		Hours					Aug. 7	1	
	15	2–4	(a)	July 20	30	(c)	Aug. 9	1	20
							Aug. 11	2	
							Aug. 18	2	
							Aug. 27	1	
							Sept. 22	2	
							Aug. 5	1	
P. O. J. 36-M	16	24	4	July 21	24	(c)	Aug. 7	1	4
							Aug. 8	1	
							Aug. 19	1	
		Days					Aug. 6	2	
	17	14	4	do	30	(c)	Aug. 7	3	6
							Aug. 11	1	
	18	28	(e) −6	Sept. 17	10	(c)	Oct. 6	3	5
							Oct. 8	1	
							Oct. 19	1	
							Oct. 1	1	
							Oct. 5	2	
	19	22	(h) −6	do	10	(c)	Oct. 6	1	7
							Oct. 7	1	
							Oct. 8	1	
							Oct. 20	1	
	20	28	(i) −6	do	10	(c)	Oct. 5	1	1

(a) The inoculum was stored at room temperature.
(b) The plants were inoculated by rubbing the extract into the young leaves by means of a glass rod.
(c) The regular method of inoculation was employed (p. 827).
(d) The plants were inoculated by injecting the extract into a rolled leaf by means of a hypodermic syringe.
(e) The regular method of inoculation was employed, but the leaf tissue was not punctured.
(f) The regular method of inoculation was employed except that a coarse needle was used.
(g) The extract was frozen within 20 minutes after being prepared, previous to storage.
(h) In storage the extract became frozen within about 24 hours after preparation.
(i) The extract was stored for 6 days at 4° C., then frozen and stored at −6°.

TABLE 1.—*Results of inoculating Black Amber sorgo and six susceptible varieties of sugarcane with mosaic virus under various conditions in 1932*—Continued

Variety	Experiment No.	Inoculum		Inoculations			Infections observed		
		Age	Storage temperature	Date	Number	Method	Date	Number	Total
		Hours	° C.						
	21	24	4	June 9	16	(c)	June 22	1	3
							June 24	2	
	22	24	4	June 11	8	(c)	June 29	1	2
							July 3	1	
	23	*Hours* 2-4	(a)	July 2	6	(c)	Aug. 5	5	5
D-74		*Days*							
	24	28	a −6	Sept. 17	10	(c)	Oct. 5	3	6
							Oct. 9	1	
							Oct 12	1	
							Oct. 20	1	
	25	22	h −6	...do.....	10	(c)	Oct. 2	1	2
							Oct. 7	1	
	26	28	i −6	...do.....	10	(c)	----------	0	0
	27	28	4	June 22	12	(c)	July 6	1	3
							July 13	1	
							July 15	1	
	28	28	4	June 24	11	(c)	July 20	1	1
P. O. J. 234	29	28	a −6	Sept. 17	10	(c)	Oct. 1	2	3
							Oct. 5	1	
	30	22	h −6	...do.....	10	(c)	Oct. 1	1	3
							Oct. 5	2	
	31	28	i −6	...do.....	10	(c)	----------	0	0
Cristalina	32	30	4	June 27	6	(c)	July 11	1	1
	33	28	a −6	Sept. 17	10	(c)	Oct. 5	2	4
							Oct. 6	1	
							Oct. 9	1	
B. H. 10/12	34	22	h −6	...do.....	10	(c)	Oct 1	1	5
							Oct. 5	2	
							Oct. 9	2	
	35	28	i −6	...do.....	10	(c)	----------	0	0

[a] The inoculum was stored at room temperature.
[c] The regular method of innoculation was employed (p. 827).
[g] The extract was frozen within 20 minutes after being prepared, previous to storage.
[h] In storage the extract became frozeu within about 24 hours after preparation.
[i] The extract was stored for 6 days at 4° C., then frozen and stored at −6°.

RELATIVE EFFECTIVENESS OF DIFFERENT METHODS OF INOCULATION

Experiment 1, Table 1, shows that sorgo may be successfully inoculated by merely rubbing the extract into the leaves without puncturing the tissue; out of 12 tests, 8 were successful. This rubbing method is not usually so efficient in transmitting the disease, however. Experiments 2 and 3, also with sorgo, show the relatively low degree of efficiency of inoculation by means of a hypodermic syringe as compared with inoculation by means of a drop of inoculum and several fine-needle punctures (regular method). Out of 12 tests, the syringe method gave only 2 infections; the regular method gave 8.

Experiments 4 and 5, also with sorgo, confirm the frequently repeated observation that merely placing inoculum on the surface of the young leaf, without puncturing, is not a successful method of inoculation. In each of these cases there were 6 tests; the regular method gave 3 infections; the same procedure without puncturing gave none. Experiments 9 and 10, with Louisiana Purple sugarcane, show similar results; the regular method gave 6 infections out of 11 inoculations, whereas 10 similar inoculations without puncturing resulted in no infection.

Experiments 7 and 8, with Louisiana Purple sugarcane, indicate that where the regular method of inoculation is employed a very fine needle gives a higher percentage of successful infections than does a coarse dissecting needle. In these two experiments the use of a very fine needle resulted in 7 infections out of a possible 13, whereas when a coarse dissecting needle was employed there were only 3 infections out of a possible 12.

INFLUENCE OF TEMPERATURE AND AGING ON INFECTIOUSNESS OF INOCULUM

Because of the preliminary nature of the study reported in this paper, the data available for any single variety of plant are not sufficiently extensive to warrant quantitative comparison of the several varieties tested. It is obvious, however, that when inoculation was made in the regular manner, all the varieties were capable of infection.

The relations between the result of inoculation and the temperature and period of storage of the inoculum may be shown if the whole series of experiments in which the regular method of inoculation was employed is divided into categories according to the preinoculation treatment given to the inoculum, without reference to either plant variety or number of repetitions. Such a classification is shown in Table 2, which comprises all the experiments listed in Table 1 except Nos. 1, 3, 4, and 8, in which the regular method was not employed. Since there is no evidence that date of inoculation or season of the year have influenced the results of these experiments, the dates of the experiments, as given in Table 1, are not repeated in Table 2. The percentages of infection for the various groups, given in the last column of Table 2, indicate approximately the corresponding relative effects of the various treatments on the infectiousness of the mosaic virus.

The mean percentages of infection for groups 1 to 4 indicate that the virulence or infectiousness of the extract used as inoculum tended to decrease with longer storage at a temperature as low as 4° C. The tests of group 1 (with nearly fresh extract) show a mean percentage of 66, those of group 2 (with extract that had been stored about a day at 4°) show a corresponding percentage of 44, and those of group 3 and of group 4 (with extracts that had been stored at 4° for about a fortnight and for about a month, respectively) show corresponding values of only 22 and 21. Infectiousness appears to have been reduced, however, only by about two-thirds (from 66 to 21 per cent) as a result of the longest storage at 4°. About two weeks of storage at 4° appears to have resulted in as much reduction as was shown when the extract had been stored nearly a month; the mean infection percentages for groups 3 and 4 are not significantly different. These results show that expressed juice of mosaic-infected sugarcane stored in open beakers or vials for several weeks at a temperature somewhat above the freezing point of water did not completely lose its infectiousness. Such storage, however, even for a single day, produced a marked reduction in the mean value of the infection percentage.

TABLE 2.—*Relative infectiousness of similar mosaic extracts (from infected sugarcane P. O. J. 234) variously treated prior to their use in making inoculations*

Group No.	Treatment of inoculum	Experiment No.	Plant inoculated	Inoculations	Infections	
				Number	*Number*	*Per cent*
1	Extract stored only about 2 to 4 hours at room temperature	5	Sorgo	6	3	50
		9	Louisiana Purple cane	11	6	55
		13	P. O. J. 36–M cane	6	5	83
		15	do	30	20	67
		23	D–74 cane	6	5	83
	Total			59	39	66
2	Extract stored about 1 day at 4° C.	2	Sorgo	12	8	67
		6	Louisiana Purple cane	6	5	83
		7	do	13	7	54
		16	P. O. J. 36–M cane	24	4	17
	Total			55	24	44
3	Extract stored 10 to 14 days at 4° C.	11	Louisiana Purple cane	5	1	20
		12	do	11	3	27
		17	P. O. J. 36–M cane	30	6	20
	Total			46	10	22
4	Extract stored 25 to 28 days at 4° C.	14	P. O. J. 36–M cane	5	3	60
		21	D–74 cane	16	3	19
		22	do	11	2	18
		27	P. O. J. 234 cane	12	3	25
		28	do	11	1	9
		32	Cristalina cane	6	1	17
	Total			61	13	21
5	Extract frozen within 20 minutes after preparation, then stored about 27 days at −6° C.	18	P. O. J. 36–M cane	10	5	50
		24	D–74 cane	10	6	60
		29	P. O. J. 234 cane	10	3	30
		33	B. H. 10/12 cane	10	4	40
	Total			40	18	45
6	Extract stored about 27 days, at −6° C., becoming frozen within first 24 hours	19	P. O. J. 36–M cane	10	7	70
		25	D–74 cane	10	2	20
		30	P. O. J. 234 cane	10	3	30
		34	B. H. 10/12 cane	10	5	50
	Total			40	17	43
7	Extract first stored 6 days at 4° C., then frozen and stored 20 days at −6°	20	P. O. J. 36–M cane	10	1	10
		26	D–74 cane	10	0	0
		31	P. O. J. 234 cane	10	0	0
		35	B. H. 10/12 cane	10	0	0
	Total			40	1	3

To secure the highest percentage of infection it appears to be desirable to use the extracted juice while fresh. Storage in an ordinary refrigerator, however, may be expected to preserve the infectiousness of this virus for a long time, despite its apparently progressive loss of virulence.

The percentages for groups 5, 6, and 7 indicate that a storage temperature somewhat below the freezing point of water preserved the virulence of the virus much more satisfactorily than did a temperature of about 4° C. As might be expected, for freezing storage it appears to be desirable to bring the extract into the frozen condition soon after its preparation, for a 6-day storage at 4° followed by a 20-day storage at −6° gave very unsatisfactory results (group 7, with mean percentage of only 3). Nevertheless, the percentage value for group 5 (with quick freezing) is only slightly—and probably not significantly—higher than that for group 6 (without quick freezing). It

may therefore be recommended that extract of mosaic-infected sugarcane intended for inoculation tests similar to those of the present study should be prepared at a temperature as low as is feasible (with cold press and vessels) and brought to a freezing storage temperature promptly. It is important to note that extract stored for nearly a month at −6° (groups 5 and 6) gave, on an average, infection percentages (45 and 43), approximately the same as that given by similar extract stored at 4° for a single day (group 2, with a mean percentage of 44). Since it appears that the freezing of the extract did not diminish its infectiousness, it might be desirable to employ freezing of the infected tissue to aid in freeing the juice from the cells, as has frequently been done when plant tissues were to be extracted for other purposes. The infected material would thus be first frozen, then thawed, ground, and pressed, and finally stored at a freezing temperature.

Although the rather broad field explored in this preliminary study and the consequent lack of extensive series of strictly comparable data make the application of detailed numerical computations and more precise analytical methods inapplicable, yet it may be interesting to note that the highest infection percentages for individual experiments are shown for experiments 13 and 23 (group 1, with fresh extract) and for experiment 6 (group 2, with extract that had been stored about 1 day at 4° C.), each of which gave 5 infections out of 6 inoculations, or 83 per cent of successful transmission of the disease. The next lower individual percentages (67 to 70) were given by experiment 15 (group 1, with fresh extract), experiment 2 (group 2, with extract that had been stored about 1 day at 4°), and experiment 19 (group 6, with extract that had been stored about 27 days at −6°).

DISCUSSION

The causative substance or virus of the sugarcane mosaic disease, when separated from the host, seems to be much more sensitive to its surroundings than are some of the other and better known plant viruses, which may be readily and successfully transmitted by the simple operation of rubbing into the bruised tissues of an uninfected plant a minute quantity of material from crushed cells of a diseased plant.

The deterioration of the juice expressed from infected plants is probably the result of a number of factors. When cells are crushed and pressed the physical, chemical, and physiological influences normally prevailing in the living cell contents must undergo great alteration; the characteristic vital coordinations are disturbed and the activities of various catalytic agents, such as the enzymes, become either retarded or accelerated. Furthermore, the relations between the extracted juice and the atmosphere, especially those connected with the interchange of oxygen and carbon dioxide, must be very different from the corresponding relations between living cell contents and the atmosphere. Moreover, bacteria or other microorganisms may become active in the expressed juice.

The many failures that have attended attempts to transmit the sugarcane mosaic by means of expressed sap, even when freshly prepared, indicate that successful transmission may depend on some specific manner of introducing the extracted sap into the healthy plant. The tissue with which the inoculum first comes in contact must serve as a suitable and receptive medium for the continuation

of the life or activity of the infective substance, or it must at least offer a vehicle for the conduction or transfer of that substance to cells that are capable of harboring it and disseminating it through the plant. In his study of the mechanics of inoculation, Brandes (*9*) pointed out that *Aphis maidis*, the only known natural vector of sugarcane mosaic, apparently causes no perceptible destruction of tissue as it feeds on the leaf of maize, although the fine setae of the insect's proboscis pass through the epidermis of the leaf, and generally past several cell layers until they penetrate the phloem. The slight surface wounding caused by the aphid seems to produce no serious injury, nor do the disease symptoms appear first at the place of puncture. As shown by the writer (*30*) and others, mere surface contact between healthy and diseased sugarcane plants does not bring about transmission, since leaves of healthy plants in the field in contact with leaves of diseased plants remained healthy indefinitely.

It seems clear that in order to produce infection, virus or infected juice must come into intimate contact with the inner cells. But artificial applications to interior tissues do not always lead to infection. According to Stevenson (*37*), Earle (*16*), the writer (*30*), and Sein (*32*), the application of diseased tissue or juice to knife-cut surfaces of internal stem tissue of healthy plants or cuttings did not produce the disease. Nevertheless, Bonazzi (*5*) reported successful inoculations through comparatively large stem wounds made with a borer, and Wilbrink (*41*) induced infection in healthy seed cuttings by cutting them with a knife that had been used to cut diseased cane. Brandes (*9*) suggested that in plants inoculated by means of aphids the phloem may be the path of transmission and that in successful artificial inoculations through mature stem tissue the infection may have been transmitted to the new shoots by way of the vascular bundles rather than through the mature and inactive parenchymatous tissue. The success or failure of inoculation experiments with this mosaic may be explained by assuming that infection is most apt to result from artificial introduction of diseased juice if the operation is so performed as to bring the inoculum into immediate contact with some special cells of the vascular bundles without undue injury either to these or to the overlying tissues.

Since this virus is systemic in the infected host plant; since the host with the virus in it continues active existence almost indefinitely, although in many cases small regions of dead or shrunken parenchyma may be observed in the stems of infected plants (*30*); and since the virus loses its potency in dead tissue, it follows that the virus is not only naturally adapted to the living cells of the host but that its virulence depends upon the conditions of living tissue.

When successful inoculations were made through the rolled leaves of a young cane plant the first visible symptoms of mosaic were generally observed only after two weeks or more, usually as much as 12 inches or more below the point of inoculation and in the second or third leaf, but not in the outermost and oldest leaf of the spindle into which inoculation had been made. At the same time first symptoms were often discernible in inner leaves of the spindle that had not been reached by the infecting instrument at all. The virus must have descended several inches through the first infected older leaf or leaves to their insertions, whence it seems to have passed into the bases of the younger leaves, moving upward with their growth until the symptoms

became visible as the leaves expanded. In this respect sugarcane mosaic is like other mosaic diseases. It may, therefore, be supposed that the virus is not at once active in the production of mosaic symptoms in an inoculated leaf, but that it passes downward in that leaf for a long distance without causing visible injury and produces visible symptoms only after it has moved upward into still younger leaves. This supposition appears to agree with observed facts. There is reason for believing that the path of descent may be in the phloem.

Whatever the method by which diseased sugarcane juice has previously been applied to healthy plants—whether it has been rubbed into wounded leaves, injected with a hypodermic syringe, or pricked in with a needle—no attempt apparently has been made to prevent access of air to the lesions. It is reasonable to suppose that the hydrostatic and gas pressures that prevail in the vessels and in the closed intercellular spaces of the healthy sugarcane leaf are somewhat lower than the gas pressure of the external air and of the substomatal and other gas-filled spaces that are in direct communication with the external air, especially when transpiration is proceeding rapidly. Under such conditions of pressure gradients, minute quantities of air would promptly enter any wound or incision made in the leaf, usually before the entrance of the liquid inoculum, no matter how promptly the latter might occur. Thus it may happen that at least some portions of the internal tissue surfaces suddenly exposed by a mechanical incision become covered with a thin gas layer before they are really reached by the diseased juice, and actual access of the latter to such gas-covered surfaces may be greatly retarded or even prevented. It is believed that these possibilities were largely avoided in the present experiments by first covering the region where inoculation incisions were to be made with a drop of the liquid inoculum, which remained in position as a water seal over the incisions until reduced and removed through evaporation and absorption; suction or diffusion of virus into the wounds continued to occur long after the completion of the stabbing operation. This expedient of making the needle stabs through a properly placed drop of diseased juice gave added assurance that the needle point was always thoroughly wetted with the liquid as it entered the foliar tissue.

Because of turgor, the hydrostatic pressure of living cells, including the elements of the phloem, is greater than that of the sap in the vessels; it exceeds also the gas pressure of the atmosphere and of the intercellular spaces. Hence some exudation may occur from living cells that happen to be penetrated by the stabbing needle. When stabbing is performed through a drop of diseased juice the sucked-in inoculum should tend to come into immediate contact with any extruded cell contents, and the complex system of surface-tension gradients thus set up should result in convection currents that would effectively mix the two liquids. This sort of mixing action would be much more rapid than simple diffusion and might continue for a long time after the operation of stabbing had been completed.

SUMMARY AND CONCLUSIONS

In this paper the present state of knowledge concerning the mosaic disease of sugarcane is reviewed and new experimental results of inoculation tests with this mosaic are presented. A new method of

inoculation is described. In inoculation tests on healthy plants of the mosaic-susceptible sorgo variety Black Amber and on the mosaic-susceptible sugarcane varieties, the extracted juice of diseased sugarcane was used as inoculum. In making an inoculation a drop of inoculum was placed in the wedge-shaped opening between the base of the youngest expanded leaf blade and the next younger leaf, recently exposed but still tightly rolled, on the same side of the false stem or leaf spindle. Through the liquid held in this wedge-shaped reservoir a very fine needle was repeatedly thrust with gentle pressure, pricking the submerged area of the rolled leaf. The pricking or stabbing was thus done under the liquid, which was allowed to remain in place till it evaporated or was absorbed. Where fresh or properly stored juice was used, high percentages of infection generally resulted within a few weeks, although the juice was never protected from the air. Except in inoculated plants, no infection was observed at any time during the investigation either in plants serving as controls or in other plants in the greenhouse.

The results obtained indicated that expressed juice of mosaic-infected sugarcane tends increasingly to lose its infectiousness if kept in open beakers or vials for a day or more at room temperature or even at a temperature of about 4° C. This tendency was largely overcome, however, when the juice was stored in open vessels at a temperature of about −6°, at which the liquid became solidly frozen within 24 hours, and so remained until thawed at room temperature just before it was used in inoculation tests. High percentages of infection were obtained when inoculations were made (by the new or "regular" method) with juice that had been stored in the frozen condition for as long as 27 days. Infectious juice that had been kept for 6 days at a temperature of 4° and was then frozen and stored for 20 days at −6° was found to have lost most or all of its infectiousness. In the light of present knowledge it may be recommended that infected juice for inoculation experiments of the sort here reported be frozen as soon as feasible after its preparation and be kept frozen until thawed for use; and also that the grinding and pressing operations by which infected juice is prepared should be performed at a temperature as low as possible.

LITERATURE CITED

(1) Allard, H. A.
1914. The mosaic disease of tobacco. U. S. Dept. Agr. Bul. 40, 33 p., illus.

(2) ———
1918. Effect of various salts, acids, germicides, etc., upon the infectivity of the virus causing the mosaic disease of tobacco. Jour. Agr. Research 13:619–637.

(3) Arendsen Hein, S. A.
1893. Vraagbord. Arch. Java Suikerindus. 1: [215]–216, illus.

(4) Beijerinck, M. W.
1898. Ueber ein contagium vivum fluidum als ursache der fleckenkranheit der tabaksblätter. Verhandel. K. Akad. Wetensch. Amsterdam (2 sect.) deel 6, No. 5, 21 p., illus.

(5) Bonazzi, A.
1926. Study on sugar cane mosaic. Science (n.s.) 64:529–530.

(6) BRANDES, E. W.
1919. THE MOSAIC DISEASE OF SUGAR CANE AND OTHER GRASSES. U. S. Dept. Agr. Bul. 829, 26 p., illus.
(7) ———
1920. ARTIFICIAL AND INSECT TRANSMISSION OF SUGAR-CANE MOSAIC. Jour. Agr. Research 19:131–138.
(8) ———
1920. MOSAIC DISEASE OF CORN. Jour. Agr. Research 19:517–522, illus.
(9) ———
1923. MECHANICS OF INOCULATION WITH SUGAR-CANE MOSAIC BY INSECT VECTORS. Jour. Agr. Research 23:279–284, illus.
(10) ——— and KLAPHAAK, P. J.
1923. CULTIVATED AND WILD HOSTS OF SUGAR-CANE OR GRASS MOSAIC. Jour. Agr. Research 24:247–262, illus.
(11) BRUNER, S. C.
1922. SOBRE LA TRANSMISION DE LA ENFERMEDAD DEL "MOSAICO" O "RAYAS AMARILLAS" EN LA CAÑA DE AZUCAR. Rev. Agr., Com. y Trab. [Cuba] 5(1):11–22, illus.
(12) CHARDON, C. E., and VEVE, R. A.
1922. SOBRE LA TRANSMISIÓN DEL MATIZADO DE LA CAÑA PER MEDIO DE INSECTOS. Rev. Agr. Porto Rico 9:9–20, illus.
(13) ———
1923. THE TRANSMISSION OF SUGAR-CANE MOSAIC BY APHIS MAIDIS UNDER FIELD CONDITIONS IN PORTO RICO. Phytopathology 13:[24]–29, illus.
(14) COLÓN, E. D.
1918. YELLOW STRIPE OF SUGARCANE. Porto Rico Insular Expt. Sta. Ann. Rpt. 1917–18:66–68.
(15) DOOLITTLE, S. P.
1920. THE MOSAIC DISEASE OF CUCURBITS. U. S. Dept. Agr. Bul. 879, 69 p., illus.
(16) EARLE, F. S.
1919. THE YEAR'S EXPERIENCE WITH SUGARCANE MOSAIC OR YELLOW-STRIPE DISEASE. Jour. Dept. Agr. and Labor Porto Rico 3:15–55.
(17) FAWCETT, G. L.
1924. EL MOSAICO O ENFERMEDAD DO LAS RAYAS AMARILLAS DE LA CAÑA. Rev. Indus. y Agr. Tucumán 15:103–111, illus.
(18) HENDERSON, R. G., and WINGARD, S. A.
1931. FURTHER STUDIES ON TOBACCO RING SPOT IN VIRGINIA. Jour. Agr. Research 43:191–207, illus.
(19) [KAMERLING, Z.]
1903. DE GELE STREPENZIEKTE DER BLADEREN. Proefsta. Suikerriet West Java "Kagok" to Pekalongan, Verslag 1902:76–81.
(20) KOBUS, J. D.
1908. VERGELIJKENDE PROEVEN OMTRENT GELE-STREPENZIEKTE. Arch. Java-Suikerindus. 16:350–374. Reprinted as Meded. Proefsta. Java-Suikerindus., No. 12, p. [319]–342.
(21) KUNKEL, L. O.
1921. A POSSIBLE CAUSATIVE AGENT FOR THE MOSAIC DISEASE OF CORN. Hawaii Sugar Planters' Assoc. Expt. Sta. Bul., Bot. Ser. 3:44–58, illus.
(22) ———
1922. INSECT TRANSMISSION OF YELLOW STRIPE DISEASE. Hawaii. Planters' Rec. 26: 58–64, illus.
(23) ———
1924. FURTHER STUDIES ON THE INTRACELLULAR BODIES ASSOCIATED WITH CERTAIN MOSAIC DISEASES. Hawaii. Sugar Planters' Assoc. Expt. Sta. Bul., Bot. Ser. 3:108–114, illus.
(24) LEDEBOER, F.
1921. GELESTREPENZIEKTE. Arch. Suikerindus. Nederland.-Indië, 29 (deel 2):1000–1001.

(25) LYON, H. L.
1914. LAHAINA CANE INJURED BY YELLOW STRIPE DISEASE. Hawaii. Planters' Rec. 10:320–323.
(26) ———
1921. THREE MAJOR CANE DISEASES: MOSAIC, SEREH, AND FIJI DISEASE. Hawaii Sugar Planters' Assoc. Expt. Sta. Bul., Bot. Ser. 3:1–43, illus.
(27) MCCLEAN, A. P. D.
1928. MOSAIC DISEASE OF SUGAR CANE. WITH SPECIAL REFERENCE TO ITS ERADICATION IN NATAL. So. African Sugar Jour. 12:483, 485, 487, 489.
(28) MCKINNEY, H. H.
1925. A MOSAIC DISEASE OF WINTER WHEAT AND WINTER RYE. U. S. Dept. Agr. Bul. 1361, 11 p., illus.
(29) MCRAE, W., and SUBRAMANIAM, L. S.
1928. A FURTHER NOTE ON THE MOSAIC DISEASE OF SUGARCANE. Agr. Jour. India 23:239–255, illus.
(30) MATZ, J.
1919. INFECTION AND NATURE OF YELLOW-STRIPE DISEASE OF CANE (MOSAIC, MOTTLING, ETC.) Jour. Dept. Agr. and Labor Porto Rico 3(4):65–82, illus.
(31) MUSSCHENBROEK, S. C. VAN.
1898. BESCHRIJVING VAN TWEE TOT DUSVERRE IN WEST-JAVA ONBEKENDE RIETZIEKTEN. SOERABAIASCHE VEREENIGING VAN SUIKER FABRIKANTEN. Circulaire No. 42. Bijlage Arch. Java Suikerindus. 1893 : [113]–118.
(32) SEIN, F. Jr.
1930. A NEW MECHANICAL METHOD FOR ARTIFICIALLY TRANSMITTING SUGAR CANE MOSAIC. Jour. Dept. Agr. Porto Rico 14:49–68.
(33) STAHL, C. F.
1927. CORN STRIPE DISEASE IN CUBA NOT IDENTICAL WITH SUGAR CANE MOSAIC. Trop. Plant Research Found. Bul. 7, 12 p., illus.
(34) ——— and FARIS, J. A.
1929. THE BEHAVIOR OF THE NEW P. O. J. CANES IN RELATION TO SUGAR CANE MOSAIC IN CUBA. Trop. Plant Research Found. Bul. 9, 12 p., illus.
(35) STEVENSON, J. A.
1917. AN EPIPHYTOTIC OF CANE DISEASE IN PORTO RICO. Phytopathology 7:[418]–425, illus.
(36) ———
1917. REPORT OF THE PATHOLOGIST. Bd. Comm. Agr. Porto Rico Rpt. 5:58–70.
(37) ———
1919. THE MOTTLING OR YELLOW-STRIPE DISEASE OF SUGARCANE. Jour. Dept. Agr. and Labor Porto Rico v. 4 (i. e., v. 3 No. 3) No. 1, p. 3–76, illus.
(38) STOK, J. E. VAN DER.
1907. VERSCHIJNSELEN VAN TUSSCHENRASVARIABILITEIT BIJ HET SUIKERRIET. Meded. Profesta. Oost-Java, ser. 4, No. 36, p. [457]–477.
(39) WALKER, N. N., and STAHL, C. F.
1926. CERTAIN GRASS HOSTS OF THE SUGAR CANE MOSAIC DISEASE AND OF THE CORN APHID CONSIDERED IN RELATION TO THEIR OCCURRENCE IN CUBA. Trop. Plant Research Found. Bul. 5, 14 p.
(40) WILBRINK, G.
1922. EEN ONDERZOEK NAAR DE VERBREIDING DER GELESTREPENZIEKTE DOOR BLADLUIZEN. Arch. Suikerindus. Nederland. Indië 30 (deel 2) : [413]–456.
(41) ———
1930. MECHANICAL TRANSMISSION OF SUGAR CANE MOSAIC. Cong. Internatl. Soc. Sugar Cane Technologists. Proc. (1929) 3:155–161.
(42) ——— and LEDEBOER, F.
1910. BIJDRAGE TOT DE KENNIS DER GELE STREPENZIEKTE. Meded. Proefsta. Java-Suikerindus. deel 2, No. 39, p. 443–495, illus.

THE EFFECT OF COOKING ON THE VITAMIN A AND C CONTENT OF FRESH AND DRIED APRICOTS[1]

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INTRODUCTION

The effect of drying on the vitamin content of fruits has been the subject of investigation in the laboratories of household science and fruit products of this station for a number of years. The A and C vitamins have been considered chiefly and the fruits studied were peaches, prunes, and apricots. As previously reported (*4*, *6*)[2] vitamin C is well retained by sulphured, dehydrated, and sun-dried fruit but is largely lost by the unsulphured dried products. In apricots a minimum of 470 parts per million of sulphur dioxide in the dried product was found to be necessary for satisfactory preservation of the antiscorbutic property. Lye dipping previous to the sulphuring was found to be necessary in the case of prunes in order to produce sufficient penetration of the protective sulphur dioxide to effect the preservation of the vitamin C in the dried product.

Vitamin A was shown (*5*) to be well retained, 40 to 100 per cent usually, by dried peaches and prunes, both sulphured and unsulphured, and less well preserved in the dried apricots, 16 to 51 per cent. The dried apricots nevertheless were fairly rich in vitamin A because of the unusual endowment of the fresh fruit. Both sulphuring and dehydration were favorable to the retention of this vitamin as well as to that of vitamin C.

The question was raised by Nelson and Jones[3] of the Bureau of Chemistry and Soils, United States Department of Agriculture, as to the applicability of these findings to the processed sulphured dried fruit usually found on the market. They reported that very little vitamin C remained in five samples of processed and resulphured apricots, although the first sulphuring was found to exert a striking preservative effect on this vitamin.

No detail was reported, and in a later summary (*3*, *p. 24*) the statement was made that—

> sulphured apricots retain their original vitamin C content remarkably well through commercial processing and subsequent storage, whereas dried unsulphured apricots are devoid of this vitamin. However, ordinary methods of cooking destroy the vitamin completely, and if the practice of sulphuring is to be of any practical significance in the preservation of vitamin C it will be necessary to devise other methods of preparing the fruit for the table.

Before the publication of these comments a series of tests had been begun in these laboratories designed to answer both questions raised, namely, (1) the effect of processing and resulphuring and (2) of cooking on the vitamins of sulphured and unsulphured dried apricots.

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[1] Received for publication Aug. 8, 1932; issued June, 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 849.

[3] NELSON, E. M., and JONES, D. B. THE VITAMIN C CONTENT OF SULPHURED APRICOTS. [Unpublished. Title in abstracts of papers presented at Cincinnati meeting of the Amer. Chem. Soc., 1930.]

PREPARATION OF THE FRUIT

The fruit was prepared as previously described (*6*), particular care being taken that the frozen fresh fruit when packed was completely deoxygenated by evacuation and refilling with nitrogen. Royal apricots from the same orchard were used in preparation of samples S1, S2, and S3 (Table 1) and, in preparing S4, fruit from an adjoining orchard was used which had been harvested at the same time. As indicated in Table 1 the last sample was subjected to the usual commercial storage for at least five months and was then processed and resulphured according to current commercial practice.

TABLE 1.—*Preparation and composition of fresh and dried Royal apricots*

Treatment of fruit	Lot No.	Method of preparation	Moisture	Net shrinkage	pH	Sulphur dioxide content
			Per cent	*Per cent*		*Parts per million*
Fresh frozen	S1	Pitted, ground, evacuated, released with nitrogen twice, and sealed and kept at —17° C.	79.4	.....	3.70	.....
Sulphured, sun-dried	S2	Pitted, sulphured 4 hours, sun-dried 9 days, ground, packed in 8-ounce tin cans, stored at 0° C.	17.5	4.01	3.40	480
Unsulphured, sun-dried	S3	Prepared as S2 but without sulphuring.	14.7	4.14	3.75	.....
Processed, sulphured, sun-dried.	S4	Prepared as S2 but held in ordinary storage at room temperature for 5 months, then processed in the usual commercial fashion, ground, packed, and stored as was S2.	24.9	3.65	3.50	1,090

THE COOKING PROCESS

The cooking of the fresh apricots was carried on in approximately 500-g lots without added water in a covered container over boiling water. The temperature of the fruit was brought to 85° C. in 15 minutes and maintained there for 5 minutes, a total cooking time of 20 minutes. The mixture was stirred twice during this interval without removing the cover. The doses of fresh fruit were weighed after cooking. The dried fruit was soaked in a small quantity of warm water for 15 minutes and was then cooked by the same method used for the fresh fruit. All dried-fruit doses were cooked separately in order to eliminate variations in water content. The ground dried apricot cooked in this fashion is quite tender and palatable. This method of brief soaking and rapid cooking of dried fruit has recently been advocated by several workers (*10*) although somewhat longer periods (15 to 20 minutes total cooking, 3 to 5 of which was at boiling temperature), than those used in this study were found desirable for cooking unbroken apricot halves. Part of the tests were made on dried fruit soaked 2½ hours in boiling water and then cooked 15 minutes over the open flame. No difference in effect upon vitamin content was noted between this and the other methods used.

EXPERIMENTAL RESULTS

THE VITAMIN C TESTS

The methods used in testing for vitamin C were those previously described. The period of feeding was maintained at 60 days, which period this laboratoy had found to be as satisfactory for vitamin C assay as 90 days (*6*).

TABLE 2.—*Vitamin C content of raw and cooked fresh apricots*

Treatment and crop	Condition	Quantity fed daily	Guinea pigs used	Average weight of animals			Average gain or loss in weight per day	Average length of period	Scurvy score
				Initial	Final	Gain or loss			
		Grams	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Days*	
Fresh frozen, 1930 crop	Raw	10	3	340	304	−36	−0.6	56	8
	Cooked		4	343	263	−80	−1.6	49	12
	Raw	15	4	327	508	181	3.2	60	0
	Cooked		7	359	417	58	1.1	51	5
	Raw	20	4	325	488	163	2.8	58	0
	Cooked		5	351	453	102	1.8	56	3
	do	30	2	352	542	190	3.8	50	0
Fresh frozen, 1929 crop	Raw	15	3	347	459	112	2.2	51	0
	do	25	3	324	452	128	2.0	64	0

As shown in Table 2 and Figure 1, 15 g of the fresh raw apricot daily afforded full protection against scurvy as indicated by the usual autopsy findings, but more than 20 g of the cooked fresh fruit was needed for similar protection. There would seem to be a consistent loss of antiscorbutic value in this fruit attributable to the amount of cooking used in these tests. This loss is apparently 30 to 50 per cent of the vitamin C activity of the fresh raw fruit.

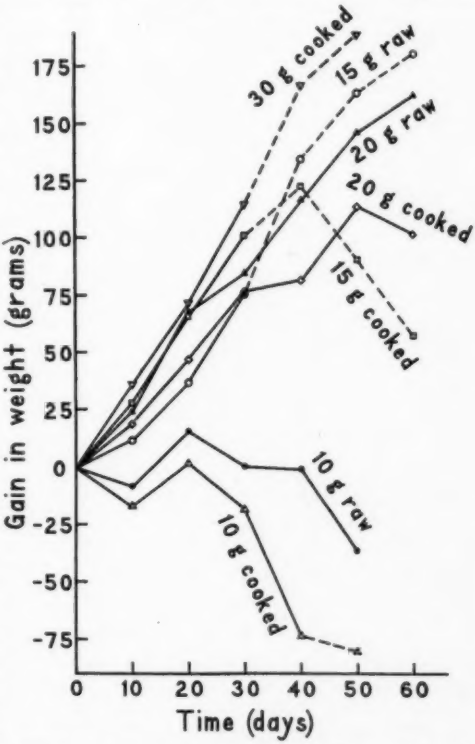

FIGURE 1.—Average growth curves of guinea pigs fed various quantities of raw and cooked fresh apricots, S1. When one or more animals in a group died or was sacrificed the curve is continued as a dotted line

Similar cooking of the sulphured dried apricots, S2, brought about less decrease in the vitamin C value. The differences in scurvy scores and in rate of growth, as shown in Table 3 and Figure 2, by the animals fed the same dosages, raw and cooked, are considerably smaller than in the case of the fresh apricots. The sulphur dioxide may exert its protective effect on the vitamin during the cooking as well as during the drying process. Since the raw, unsulphured, dried fruit, as was found in a number of cases pre-

viously, is too poor in the antiscorbutic property to afford any noticeable protection it was not deemed worth while to make the test with cooked unsulphured dried fruit (*4*).

TABLE 3.—*Vitamin C content of raw and cooked, sulphured and unsulphured, dried apricots*

Treatment and crop	Lot No.	Condition	Quantity fed daily: Dried fruit	Quantity fed daily: Equivalent in fresh fruit	Guinea pigs used	Average weight of animals: Initial	Average weight of animals: Final	Average weight of animals: Gain or loss	Average gain or loss in weight per day	Average length of period	Scurvy score	Retention of vitamin C of fresh raw fruit (per cent)
			Gms	*Gms*	*Number*	*Gms*	*Gms*	*Gms*	*Gms*	*Days*		
Sulphured sun-dried, 1930 crop.	S2	Raw	2	8	4	348	305	−43	−0.9	49	8	Raw and cooked, 94–100.
		do			5	312	368	56	0.9	60	5	
		Cooked	3	12	3	328	353	25	0.4	56	7	
		Raw	4	16	7	319	428	109	1.8	60	1	
		Cooked			5	329	420	91	1.5	60	2	
		Raw	5	20	4	314	487	173	2.9	60	1	
		Cooked			4	309	444	135	2.2	60	2	
After storage at room temperature for 1 year.	S2	Raw	5	20	3	328	253	−75	−1.3	60	8	Raw and cooked, about 40.
		Cooked			6	315	257	−58	−1.3	44	9	
		do	8	32	4	322	339	17	0.3	57	7	
Unsulphured sun-dried, 1930 crop.	S3	Raw	10	41	1	310	206	−104	−3.0	34	20	0.
Unsulphured dehydrated, 1929 crop.		do	15	62	3	353	227	−126	−3.8	33	13	0.
Unsulphured dehydrated, 1928 crop.		do	1–10	4.5–45	8	326	230	−96	−3.0	32	20	0.
Sulphured sun-dried, processed, 1930 crop.	S4	do	2	7	3	319	217	−102	−3.3	31	9	Raw and cooked, about 50.
		Cooked			2	342	257	−85	−2.0	43	12	
		Raw	3	11	5	319	235	−84	−2.5	34	16	
		Cooked			3	353	267	−86	−2.5	35	12	
		Raw	4	14	4	329	293	−36	−0.6	53	10	
		Cooked			4	328	327	−1	±0.0	53	11	
		Raw	5	18	10	329	352	−23	0.3	57	7	
		Cooked			2	348	273	−75	−1.4	54	15	
		Raw	8	29	6	338	391	53	0.9	60	4	
		Cooked			4	339	387	48	0.8	60	4	
		Raw	10	36	1	330	437	107	1.8	60	2	

After storage at room temperature for one year the vitamin C content of the sulphured dried fruit, S2, was tested again, both raw and after a longer cooking process. The fruit which had been ground and kept in 8-ounce tin cans, was soaked for 2½ hours in three times its weight of water which was boiling when it was added to the fruit, and was then cooked over the open flame for 15 minutes. The doses could be measured accurately in equivalents of the raw material by comparing the weights of the cooked and raw fruit. As shown in Table 3 a considerable loss of vitamin C occurred during the long storage of this fruit, a loss possibly correlated with the corresponding loss of sulphur dioxide during storage now under investigation in the fruit products laboratory (*8*).

The same influences which produce oxidation of the sulphur dioxide may operate to oxidize the vitamin. At any rate, after storage at room temperature for one year or more, 5 g of the sulphured, dried apricots either cooked or raw did not protect the animals as nearly completely from scurvy as when the fruit was first tested, but appeared

equivalent antiscorbutically to only 2 g of the original lot. Even 8 g cooked provided only partial protection, about equivalent to that afforded by 3 g of the fruit originally tested. Thus apparently only 40 per cent of the antiscorbutic property was retained after long storage.

The sulphur dioxide content of this stored specimen was tested and found to be only 330 p. p. m. as compared with 480 p. p. m. when the fruit was first prepared. This loss of 31 per cent of the sulphur dioxide may be compared with the loss of 60 per cent of antiscorbutic value. A further test was made of the sulphur dioxide remaining in the stored fruit after the cooking process above described (15 minutes rapid boiling over the open flame). It was found that less than half the quantity present in the raw sample remained, or only 160 p. p. m., a loss of 52 per cent due to cooking. If there is any advantage to be gained by decrease in the sulphur dioxide ingested with dried fruit this decrease during cooking may be looked upon as favorable, particularly since it seems to be accompanied by no concomitant loss of vitamin.

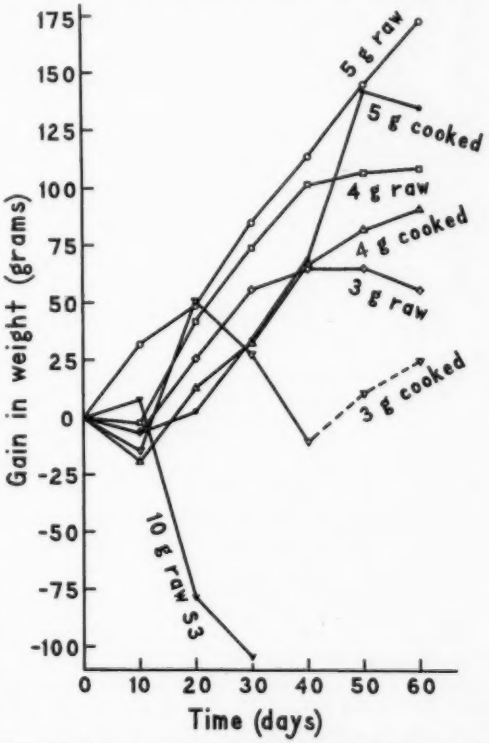

FIGURE 2.—Average growth curves of guinea pigs fed various quantities of raw and cooked sulphured, dried apricots, S2 and raw, unsulphured apricots S3

The processed resulphured fruit, S4, was found to be less well endowed with vitamin C than the unprocessed specimen S2, although with 8 and 10 g doses both raw and cooked nearly complete protection was afforded. Here again as in S2, shown in Figures 2 and 3 and Table 3, the cooking apparently had little destructive effect upon the vitamin.

Fifteen grams of the raw fresh apricot may be considered the minimum protective dose, and 25 g a reasonable estimate of the minimum dose of the cooked fresh fruit. Since the equivalent of 16 g raw or cooked fed as S2 appears to be the minimum dose of the sulphured dried fruit which furnishes practically complete protection, full retention of the vitamin value of the fresh apricot is shown by both the raw and cooked sulphured dried product.

Since at least 8 to 10 g of the processed fruit, S4, equivalent to 29 to 36 g fresh, must be fed to obtain a fair degree of protection, apparently one-half the original antiscorbutic activity of the fresh fruit is lost. The long storage period at room temperature to which this sample had been subjected may be of more importance than the processing in explaining this loss, a supposition borne out by the actual loss in both vitamin C and sulphur dioxide observed in the S2 sample tested after one year in such storage. Storage at 0° C., the ordinary practice for the dried fruit tested in this laboratory, has been found in the case of several kinds of fruit to permit little or no loss of either vitamins C or A. Freezing storage would therefore appear to be desirable for dried fruits in order to conserve these vitamins.

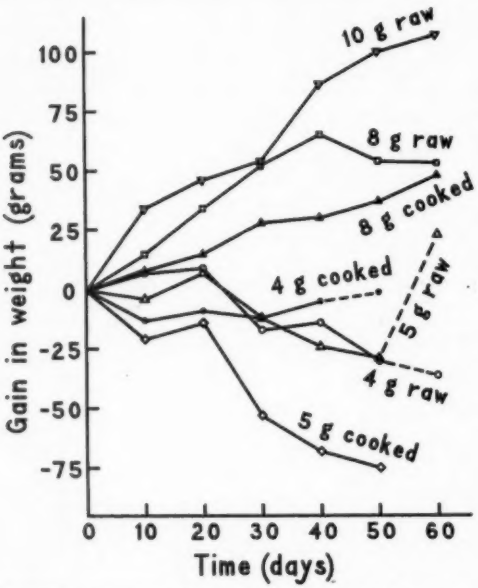

FIGURE 3.—Average growth curves of guinea pigs fed various quantities of raw and cooked processed, sulphured apricots, S4

TABLE 4.—*Vitamin A content of raw and cooked fresh apricots*

Treatment, crop, and water content	Condition	Quantity fed daily	Rats used	Average weight of animals			Average length of period	Average gain in weight per week
				Initial	Final	Gain		
		Mg	*Number*	*Grams*	*Grams*	*Grams*	*Days*	*Grams*
Fresh frozen, 1930 crop, 79.4 per cent water.	Raw	15	4	73	81	8	24	2.3
	Cooked		6	68	89	21	45	3.2
	Raw	25	4	72	92	20	44	3.2
	Cooked		6	77	113	36	47	5.3
	Raw	30	12	68	105	37	55	4.7
	Cooked		9	54	89	35	45	5.4
	Raw	40–50	9	60	110	50	56	6.3
	Cooked	50	6	71	133	62	56	7.7
Fresh frozen, 1928 crop, 82.9 per cent water.	Raw	25	3	92	151	59	56	7.3
	do	50	9	59	131	72	56	9.0
	do	100	8	71	148	77	56	9.6
	do	150	3	68	149	81	56	10.2
	do	200	3	79	181	102	56	12.8

TABLE 5.—*Vitamin A content of raw and cooked, sulphured and unsulphured, dried apricots*

Treatment and water content	Lot No.	Condition	Quantity fed daily		Rats used	Average weight of animals			Average length of period	Average gain in weight per week	Retention of vitamin A of fresh fruit (per cent)
			Dried fruit	Equivalent in fresh fruit		Initial	Final	Gain			
			Mg	*Mg*	*Number*	*Gms*	*Gms*	*Gms*	*Days*	*Gms*	
Sulphured sun-dried, 17.5 per cent water.	S2	Raw	22.5	90	4	55	96	41	50	5.7	Raw, 26–41. Cooked, 25–33.
		Cooked			10	90	141	51	56	6.4	
		Raw	30.0	120	7	87	145	58	54	7.5	
		Cooked			10	117	161	44	53	5.9	
		Raw	37.5	150	4	75	130	55	56	6.9	
		Cooked			5	71	143	72	56	9.0	
Unsulphured sun-dried, 14.7 per cent water.	S3	Raw	22.5	93	7	107	134	27	50	3.8	Raw, 18–24. Cooked, 9–20.
		Cooked			10	111	132	21	48	3.0	
		Raw	30.0	124	14	102	141	39	55	4.9	
		Cooked			10	121	153	32	46	4.9	
		Raw	37.5	155	3	91	108	17	32	3.8	
		Cooked			3	109	127	18	53	2.4	
Processed sulphured sun-dried, 24.9 per cent water.	S4	Raw	22.5	82	8	106	122	16	51	2.2	Raw, 18–29. Cooked, 18–23.
		Cooked			7	110	135	25	56	3.1	
		Raw	30.0	109	10	99	142	43	55	5.5	
		Cooked			8	104	146	42	53	5.6	
		Raw	37.5	137	9	99	153	54	56	6.8	
		Cooked			9	105	149	44	56	5.5	
Sulphured sun-dried, 1928 crop, 17.5–19 per cent water.	----	Raw	20.0	96	8	69	109	40	56	5.0	Raw, 17–21.
		do	25.0	118	4	87	151	64	56	8.0	
		do	30.0	143	7	69	123	54	56	6.8	

THE VITAMIN A TEST

The methods used in testing for vitamin A were the same as have been previously used in this laboratory (*5*). The cooked doses of both fresh and sulphured dried fruit appeared to support somewhat better growth (Tables 4 and 5 and fig. 4) than did the raw. This may be due to better intestinal absorption of the former, possibly through the destruction of the oxidases known to be present in fresh apricots (*2*) or to some other factor by which better carotene absorption is assured, a process upon which vitamin A utilization appears to depend (*1*). The vitamin A value of the fresh apricots of the 1930 crop as shown in Table 4 is somewhat lower than that found for the 1928 crop. The sulphured dried fruit, S2, showed values (Table 5) quite similar to those of the corresponding 1928 sample. The processed fruit, S4, appeared to have a slightly decreased vitamin A value and the unsulphured, dried apricot, S3, showed somewhat greater loss when compared with both the fresh and the sulphured dried products. The percentage retention by the dried samples of the vitamin A of the fresh fruit was calculated by comparison of the dose (in terms of fresh-fruit equivalent) required to provide the same amount of growth by the fresh and dried fruit. Thus, since a 90-mg equivalent of S2 raw and 30 mg of fresh raw apricot, S1, allow an average weekly gain of 5.7 and 4.7 g, respectively, S2 may be said to retain about 33 per cent of the vitamin A activity of the fresh apricot. Of course such comparisons can be made only roughly and no claim is put forward for finely drawn distinctions among the percentage retentions given in Table 5. Nevertheless it seems reasonable to assume that 26 to 41 per cent of the vitamin A is preserved in

the raw sulphured sun-dried fruit, S2, and 25 to 33 per cent in the cooked samples of the same lot; 16 to 28 per cent in the raw unsulphured dried sample, S3, and 9 to 20 per cent of the cooked; 18 to 38 per cent in the raw processed sulphured fruit, S4, and 18 to 23 per cent of the cooked. (The cooked fresh fruit is compared with the cooked dried samples and the raw fresh with the raw dried.) The order of decreasing retention value appears to be S2, S4, S3, for both raw and cooked doses as illustrated in Figure 4, and some loss due to cooking appears to occur in the unsulphured lot, S3.

Attention is called to the unusually high vitamin A content of apricots, a characteristic undoubtedly connected with their pigmentation by carotene. A comparison of the carotene and vitamin A values of these apricot samples was made and found to exhibit a close correlation (*7*). In terms of the Sherman and Munsell (*9*) units, the raw

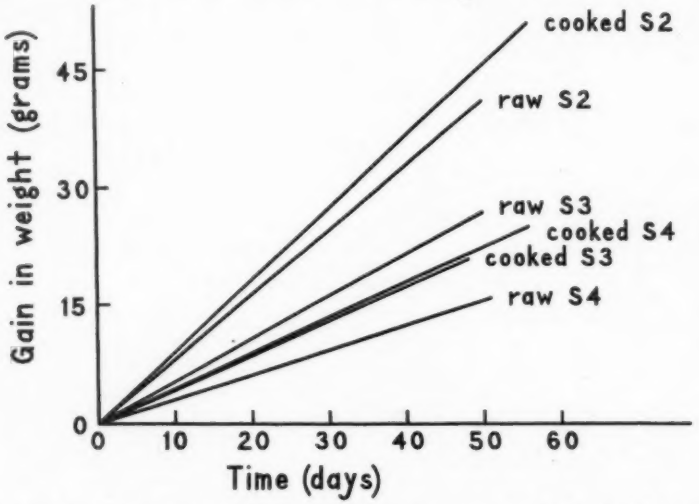

FIGURE 4.—Average weight increases of rats fed 22.5 mg daily of raw and cooked dried apricots: Sulphured sun-dried, S2; unsulphured, sun-dried, S3; processed, sulphured, sun-dried, S4

fresh apricots contain 18,000 units per pound, the cooked fresh apricots, 30,000 units per pound, the sulphured and unsulphured dried fruit about 20,000 units per pound. These values are about the same as those for butter, cheese, cream, and spinach and are exceeded only by liver, cod liver oil, and certain other liver oils.

In terms of the international vitamin A unit recently adopted by the Leagues of Nations Health Organization, 0.001 mg carotene, the fresh apricot, S1, contains 18 to 21, S2 55 and S3, 51 units per gram (*7*). These calculations are made from the colorimetric determination of carotene in the samples. If the biological test for carotene be used and the growth value of 0.001 mg carotene in vitamin A depleted rats be accepted as 6 to 8 g per week for eight weeks (*7*) the value for the fresh apricots becomes 22, for the sulphured dried 33, for the unsulphured dried less than 33, and for the processed sulphured dried 27 units per gram. The corresponding figures in units per pound are close to

10,000 for the fresh fruit, 15,000 for S2, something less than 15,000 for S3, and more than 12,000 for S4.

It seems desirable to express the vitamin A value of foods in terms of the international unit but as may be seen in this case it is apparently not yet safe to attempt to use colorimetric instead of biological methods for assay.

SUMMARY

The cooking of fresh apricots, preserved by freezing, reduced their vitamin C content approximately 30 to 50 per cent. Similar cooking of sulphured dried apricots of the same origin had less destructive effect upon this vitamin. It is suggested that the sulphur dioxide in the dried fruit may protect the vitamin C during the cooking as well as during the drying process.

The unsulphured dried fruit was again found to be lacking in antiscorbutic activity, but the dried sulphured product appeared to retain the full vitamin C value of its fresh fruit equivalent.

Commercially stored, processed, and resulphured dried apricots were found to have lost approximately one-half the antiscorbutic property of the fresh fruit but to have retained the remaining vitamin C throughout the cooking process.

Storage of the sulphured fruit at room temperature in closed cans for one year produced a loss of 60 per cent of its vitamin C and 31 per cent of its sulphur dioxide. Soaking this fruit in water for 2½ hours and then boiling it vigorously for 15 minutes made no change in its vitamin C content but occasioned the loss of approximately one-half of its remaining sulphur dioxide.

The vitamin A value of the cooked fresh fruit was found to be somewhat greater than that of the corresponding raw fruit. This is ascribed to better intestinal utilization of the former, possibly because of enzyme destruction or because of better carotene absorption.

Little change in the vitamin A value of the dried fruit samples was brought about by cooking, but the losses caused by the drying process amounted to 59 to 74 per cent in the sulphured, 76 to 82 per cent in the unsulphured, and 71 to 82 per cent in the processed fruit.

Attention is called to the extraordinarily large vitamin A content of both fresh and dried apricots which are equal in this respect to good butter, cheese, cream, and spinach. The number of international units of vitamin A (0.001 mg carotene) per pound is calculated to be about 10,000 for the fresh raw apricots and 12,000 to 15,000 for the dried fruit.

LITERATURE CITED

(1) Ahmad, B.
1931. The fate of carotene after absorption in the animal organism. Biochem. Jour. 25:[1195]–1204, illus.

(2) Fong, W. Y., and Cruess, W. V.
1929. The effect of pH value on the inactivation temperature of fruit oxidase. Plant Physiol. 4:537–541.

(3) Knight, H. G.
1931. Report of the chief of the bureau of chemistry and soils. U. S. Dept. Agr., Bur. Chem. and Soils Rpt. 76 p.

(4) Morgan, A. F., and Field, A.
1929. The effect of drying and of sulfur dioxide upon the antiscorbutic property of fruits. Jour. Biol. Chem. 82:579–586.

(5) ——— and Field, A.
1930. Vitamins in dried fruits. II. The effect of drying and of sulfur dioxide upon the vitamin A content of fruits. Jour. Biol. Chem. 88:9–25.

(6) MORGAN, FIELD, A., and NICHOLS, P. F.
1931. EFFECT OF DRYING AND SULPHURING ON VITAMIN CONTENT OF PRUNES AND APRICOTS. Jour. Agr. Research 42:35–45.
(7) ——— and MADSEN, E. O.
1933. A COMPARISON OF APRICOTS AND THEIR CAROTENE EQUIVALENTS AS SOURCES OF VITAMIN A. Jour. Nutrition 6:83–93, illus.
(8) NICHOLS, P. F., and CRUESS, W. V.
1932. LOSS OF SULPHUR DIOXIDE IN STORAGE. Indus. and Engin. Chem. 24:661–665.
(9) SHERMAN, H. C., and MUNSELL, H. E.
1925. THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Amer. Chem. Soc. 47:1639–1646, illus.
(10) THOMPSON, H. B., and BURK, G. C.
1929. THE INFLUENCE OF HEAT UPON WATER ABSORPTION OF CERTAIN DRIED FRUITS. Jour. Home Econ. 21:593–598.

INSECTS COLLECTED ON APPLE BLOSSOMS IN WESTERN NEW YORK [1]

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INTRODUCTION

In a study of insects in relation to the pollination of fruit blossoms in any district, one of the first questions which arises is how many and what varieties of insects are naturally available for this important work. It might be expected that if, in any given area, the number of trees in bloom at any one time is large, the number of individual insects per tree will be smaller than in an area with few trees, unless the district of large orchards happens to be one unusually suitable for the propagation of the species of insects which pollinate the blossoms. It is recognized by horticulturists that a lack of pollinating insects results either in the setting of few fruits or in defective pollination, so that not all five parts of the apple contain seeds, and the fruit is either defective or drops before ripening. It is well known that in many of the commercial fruit areas there is a sparsity of insects. In order to get a more accurate picture of the insect population of one fruit area of New York State, collections were made in the spring of 1931 from apple trees in a section of Monroe County. The results of this work are briefly recorded in this paper.

Monroe County, N. Y., is in about the center of the western New York fruit belt lying along the south shore of Lake Ontario. The part of the county in which collections were made lies in the former glacial-lake bed on flat land, the only variation in relief being that where the southernmost orchards were located the land surface is slightly more rolling. The most dense areas of fruit lie nearest the lake, and within a mile or two of the lake there is a pronounced retardation of the season in spring due to the influence of the large body of water. This is indicated by the considerably later blooming of the trees in orchard A in this work. In the county apple growing predominates, and as a rule the orchards receive excellent care. Because of the high valuation of the land, the amount of waste land is not great, but more waste land is found nearer the southernmost orchards, which are outside the typical fruit belt. Aside from orcharding, mixed farming and dairying are practiced, and near the city of Rochester there is considerable truck farming.

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[2] The writer is indebted to the following persons for the identification of the insects collected: The solitary bees, to Grace Sandhouse, of the U. S. National Museum, and the other Hymenoptera, to P. P. Babiy; the Diptera, to O. A. Johannsen; the Lepidoptera, to W. T. M. Forbes; and insects of other orders (usually not important for pollination), to Doctor Babiy.

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METHOD OF PROCEDURE

Collections [3] were made in seven orchards, hereafter referred to as orchards A, B, C, D, E, F, and G. Honeybees were not collected, but all other insect visitors to apple blossoms were taken whenever found, whether or not they may be considered valuable in pollination. Work was begun in each orchard practically as soon as any blossoms were open, and collection each day was begun as soon as insects visited the flowers and ended either with the oncoming of darkness or because of rain or some such meteorological phenomenon which stopped insect flights for the day. No night collections were made, since during the entire period the temperatures at night were too low for insect flight.

Not all the collectors were able to spare time to work during the full period of apple blossoming, and changes were made in the personnel in the several orchards from time to time. Six of the orchards were in bloom at one time, but the one nearest the lake (orchard A) came into bloom just after the trees in all the other orchards had dropped their petals. In orchard A collections were made for two days only, just after the blossoms opened, and to compensate for the shorter time, four collectors worked there the first day and two the second day, until stopped by rain. The number of hours in which collections were made in each orchard was not the same, and the data on the hours of work and the number of collectors are given later in this paper. The total man-hours of collecting for all seven orchards was 459.

Each hour's catch in each orchard was placed in a separate capsule, with a record of the locality and time. In each orchard hourly records were made of temperature, condition of the sky as to cloudiness, approximate wind velocity, and other meteorological phenomena which could be recorded without the use of instruments. It was thus possible in some degree to correlate the prevalence of the different species with conditions existing at the time of the visits to the blossoms. Temperatures were taken in the shade, whereas, of course, the insects were usually flying in the warmth of the sunshine when the sky was clear, but even a record of shade temperature gives some idea of the temperature conditions under which the insects visited blossoms. The estimates of the different collectors regarding light conditions and the degree of cloudiness were too variable to make the data useful in correlations. This is especially to be regretted because of the noticeable effect of sunshine on the flight of insects, especially solitary bees.

After collection was completed the insects were pinned, and with each individual insect was placed a record of the time and place of its capture.

BRIEF DESCRIPTION OF THE ORCHARDS

The part of orchard A, where the most collecting was done, consists of 7 acres of MacIntosh apple trees. Adjacent on the north are 3 acres of Rome Beauty trees, on the south is a mixed orchard of older trees, and on the west is a larger orchard of older Baldwin trees. The

[3] The collections were made by W. E. Blauvelt, F. G. Butcher, Chi-tung Chen, P. W. Claassen, C. R. Crosby, G. F. MacLeod, W. D. Mills, W. A. Rawlins, G. H. Rea, G. W. Simpson, D. O. Wolfenbarger, and the author.

trees are in excellent vigor. The set of fruit in 1931 was rather light, except on trees adjacent to the Rome Beauty trees, which came into bloom before the petals had fallen from the MacIntosh trees. The south side of the MacIntosh block bore a heavy crop in 1930, which may have reduced the crop of 1931. In the MacIntosh block insects (including honeybees) were rather scarce during the first part of the blooming period, although nectar was observed in the blossoms. Later on the honeybees were observed to be working well. Large branches from blossoming Oldenburg trees were placed in this orchard to provide pollen for the cross-pollination of the MacIntosh blossoms, and during the two days of collection in this orchard insects were more abundant on these branches than on the trees. During the first day a heavy catch of *Halictus provancheri* was made from one side of an Oldenburg tree in the old orchard to the south. There is little waste land near this orchard. This orchard is in the northwest corner of the county within sight of the lake.

Orchard B consists of 10 acres of mixed varieties (19 in all), not in the best of vigor. The trees are about 20 years old. About one fourth of the trees did not bloom in 1931, and about half bore practically no fruit. The set of fruit on some trees was good. Part of the orchard appears to be poorly drained, and there was small terminal growth. Control of injurious insects is not especially good. Directly across the road from this orchard is a neglected pear orchard in old sod, only a few trees of which bore fruit in 1931. This block of pear trees was visited abundantly by *Eristalis arbustorum*, and the pear blossoms appeared to attract these insects more than did the apple blossoms across the road which were in bloom at the same time. The insects had been breeding in a polluted ditch along the roadside between the two orchards. There is some waste land and meadow land near this orchard, which is in the northwest corner of the county about 2 miles from the lake.

Most of the collecting in orchard C was done in a block of about 15 acres of old trees, the varieties being Baldwin, Rhode Island Greening, Twenty Ounce, and Oldenburg. Adjacent to the block of old trees is a block of about 25 acres of younger trees in which some collections were made. Drainage is poor, and insect and disease control is not satisfactory, being attempted solely by the applications of dusts. The set of fruit in the old orchard was good and in the young orchard only fair. A few bouquets of blossoms of other varieties were used in this orchard but showed no local effect on the set of fruit. Surrounding these orchards are many other blocks of apple trees, the orchards being located in the midst of abundant plantings. There is little waste land, but there is a wood lot to the north of the young orchard. This orchard lies east of orchard B and is about 1½ miles from the lake.

Orchard D consists of two blocks on opposite sides of the road, separated by cultivated fields. In one block is a solid planting of Northern Spy about 20 years old, in good vigor and well sprayed. In this block the blossoms were pollinated by hand and the set of fruit in 1931 was good, but this can not properly be attributed to insect visitors. Adjacent on the east is a block of woodland, and on the south are open fields, partly under cultivation. The other block consists of Baldwin trees about 50 years old, the block being somewhat

thinned by loss of trees. There is an adjoining wood lot on the west and some open fields near by. More collections were made in the Baldwin block than from the Northern Spy. The set of fruit on the Baldwin was good in 1931. This orchard lies south of orchard C and is about 2½ miles from the lake.

Orchard E was merely a row of eight apple trees along a roadside, south of the densely planted fruit area. The trees, which are in a totally neglected condition and in poor vigor, are surrounded by meadows and open fields with wide fence rows. Within a mile in each direction there were not to exceed 25 apple trees and not much other attraction for the insects that habitually visit blossoms. This group of trees was selected for the purpose of comparing their condition with that of trees in the typical fruit area. These trees probably did not produce a perfect apple in 1931. This group of trees is about 15 miles directly south of orchard D.

Orchard F consists of about 5 acres of mixed varieties, interplanted. The trees are in good vigor and color and are making good growth. The Northern Spy and Baldwin trees in this orchard did not bloom, but other varieties bloomed abundantly. The orchard is surrounded by woods, waste land, open fields, and other fields under cultivation. There is an old orchard of a few trees adjacent, from which collections were not made. Orchard F lies outside the fruit area and was selected for this reason. The set of fruit in 1931 was good for the varieties which bloomed. This orchard is 2 miles south of orchard E.

Orchard G consists of about 50 acres, about 20 acres of which are Rhode Island Greening, with only occasional other varieties interspersed. The remainder consists of interplanted Baldwin and MacIntosh trees. The trees were formerly neglected and were in poor vigor but have recently been brought to much better condition. This commercial orchard was selected because there are no other orchards near it, and there is considerable waste land, swamp land, and woodland near by. These trees bore heavily in 1930, which may have had some effect on the 1931 crop. This orchard was remarkable for the sparsity of all insects in the spring of 1931, only 32 insects being taken in 53 hours of collection, 3 of which are valueless for pollination. Honeybees were not abundant but doubtless outnumbered all other insects 100 to 1. The Baldwin trees bore a fair set of fruit on those trees that were in good vigor. The set on MacIntosh and Rhode Island Greening trees was practically nothing, except for a few apples on a small number of Rhode Island Greening trees which immediately adjoined some Rome Beauty trees that bloomed at the same time. This orchard is in the southeastern part of the county and outside the fruit belt.

INSECTS COLLECTED

While the weather during the apple-blossoming period was cold at night and rain interfered with insect flight on some days, there were also some days and parts of days which were excellent for insect flight. In general there were no unusual weather conditions during this season, so that the small total number of insects captured can not be attributed to unfavorable weather.

The outstanding result of this work was the small number of individual insects captured. While the several orchards differed in the number of insects and in the relative abundance of different species,

in none of them was the insect population abundant. This applied to the orchards in the typical fruit area to a more marked degree than to those south of the fruit area, but orchard G, also south of the fruit belt, was the least well provided with wild insects.

In the 459 man-hours of collection only 3,555 individuals were taken, an average of only 7.75 insects per man per hour. The number of identified species of insects is 145, but a number of specimens were taken for which specific determinations are not now available. This may be considered as a fair representation of the number of species but not of the number of individual insects. During many hours of the collecting period it was impossible to find a single insect on the apple blossoms, their absence being due either to cool weather, lack of sunshine, or to the scarcity of the insects. Although this was the first year in which systematic collections were undertaken in Monroe County, yet from the casual observations of earlier years and from the general experience of those who have visited these orchards, the small number of insects collected is not surprising.

The most abundant wild insects visiting apple blossoms were flies (Diptera) and solitary bees (Hymenoptera). In the collections of 1931 the Diptera somewhat outnumbered the Hymenoptera, as will be seen from the tables which follow. In addition, some Lepidoptera were taken, chiefly of one species, and there were also some Coleoptera and other insects which play little, if any, part in pollination. The Lepidoptera, Coleoptera, Hemiptera, and Neuroptera are not here recorded. While probably all the Hymenoptera, with the exception of the wasps, are helpful in pollination, this is not true of all the Diptera. Some of the smaller flies can not be considered as valuable for pollination. Of the 2,056 Diptera captured, probably at least 200 individuals are too small to be of much value to the fruit grower.

DIPTERA

In Table 1 are listed all Diptera captured, and for each species is given the number of individuals taken in each orchard. The prevalence of *Eristalis arbustorum* has already been mentioned in the description of orchard B. A considerable number of individuals of this species are listed in the table under "Miscellaneous," these being the flies taken in the pear orchard across the road from orchard B. If individuals of Eristalis had been taken as often as possible, the number for orchard B would have been many times as great, but for most of the collecting period an effort was made to find other species.

TABLE 1.—*Diptera collected and frequency of each species, by orchards*

Species and sex	Frequency of each species by orchards indicated							Miscellaneous	Total
	A	B	C	D	E	F	G		
Aedes sp.	1			3		5			9
Camptocladius byssinus			1		1	7			9
Psectrocladius sp., females			2	1					3
Cricotopus bicinctus					1				1
Cricotopus trifasciatus						1			1
Tanytarsus sp.			1		2	1			4
Chironomus aberrans, females					2	5			7
Chironomus sp., females	4		8	1		33			46
Chironomus sp., males			2			18			20
Chironomus spp.			5	4	4	3			16
Sciara sp., females			6		1	15			22
Sciara sp., males			1			1			2

TABLE 1.—*Diptera collected and frequency of each species, by orchards*—Continued

Species and sex	Frequency of each species by orchards indicated							Miscellaneous	Total
	A	B	C	D	E	F	G		
Bibio xanthopus, females	4	1	1		1				7
Scatopse notata		1							1
Simulium venustum			2	2	1	2			7
Stratiomyia sp						1			1
Tabanus lasiophthalmus						3			3
Rhamphomyia spp				1	6				7
Toxomerus marginatus, females	2	2	1						5
Toxomerus marginatus, males			1						1
Pipiza pisticoides			1			1	1		3
Platychirus hyperboreus, females					1				1
Platychirus hyperboreus, males				1					1
Platychirus peltatus, males							1		1
Melanostoma mellinum, females					1	2			3
Melanostoma obscurum, females	2		3	18		4			27
Melanostoma obscurum, males				2					2
Syrphus americanus, females		2	10	2	5	1			20
Syrphus, americanus, males	1	1							2
Syrphus arcuatus, females			1	1		1			3
Syrphus arcuatus, males			1			1			2
Syrphus ribesii females	2		9	3	3	5	1	1	24
Syrphus ribesii, males				2					2
Syrphus torvus, females	1	4	16	7	4	6	3	1	42
Syrphus torvus, males		1	12	15	3	7	1		39
Allograpta obliqua, females	2	1	1	2	1				7
Sphaerophoria cylindrica, females	4	7	12	6	2	19	2	1	53
Sphaerophoria cylindrica, males	10	6	15	6		21		4	62
Eristalis arbustorum, females	9	172	80	6	2	10	1	151	431
Eristalis arbustorum, males	15	111	65	15		5		102	313
Eristalis bastardi	2		5	1					8
Eristalis dimidiatus		1	2	2		3	1		9
Eristalis flavipes	3			2					5
Eristalis tenax	5	2	3		1	2		1	14
Helophilus hamatus, females	1					1			2
Helophilus similis, females		2	1	2				2	7
Syrilla pipiens, females			1			1			2
Syrilla pipiens, males			2			1			3
Criorhina verbosa, females						1			1
Myopa vesiculosa					2	1			3
Tachinidae spp	1	1	10	2	17	9	1		41
Sarcophaga sp	1	3	10	2	10	11		1	38
Phormia regina					4	15			19
Protophormia terrae-novae					1				1
Cyanomyia cadaverina	2		1		1	1			5
Calliphora vomitoria			1	1	1	3			6
Lucilia caesar	3	1	1	1	1				7
Lucilia sericata	2			2	1	2			7
Pollenia rudis	1	1	44	6	13	34	1	2	102
Cryptolucilia caesarion			1	1		1			3
Pyrellia cyanicolor				3	1	17			21
Morellia micans			1		5	6			12
Muscina assimilis					1		1		2
Muscina stabulans			2	1	9	3			15
Myospila meditabunda		1	2		10	4			17
Fannia canicularis, females			7		2	9			18
Fannia canicularis, males		3	14		1	10	1	1	30
Hylemyia brassicae, males	1	5	47	6	5	7		1	72
Hylemyia calicrura, males	5	5	17	9	9	32	2	2	81
Hylemyia sp., females	5	3	81	22	15	35	4	9	174
Hylemyia sp., males	3		21	3	3	11		4	45
Pegomyia affinis, males						1			1
Scatophaga furcata, females			2			1	1	1	5
Scatophaga furcata, males				3					3
Scatophaga stercoraria, females			4			1		1	6
Scatophaga stercoraria, males			4	2	2	1		1	10
Sphaerocera pusilla					1				1
Sphaerocera subsultans						7			7
Leptocera sp						2			2
Lonchaea polita	1		3			1		1	6
Sepsis pectoralis			2			1			3
Sepsis sp., females			3			1			4
Nemopoda cylindrica						3			3
Piophila sp			1		3			2	6
Chloropisca glabra	2								2
Crassiseta costata						5			5
Botanobia nitidissima						2			2
Mallochiella glabra			3		1	6			10
Total	95	337	553	169	161	430	22	289	2,056

Of the Diptera found on apple blossoms the syrphid flies (Syrphidae) are most valuable, and 1,095 individuals of this family are included in the collection. These flies are fairly well distributed in the different orchards, except in orchard E and in orchard G in which, as already mentioned, there was a general lack of all insects.

No effort has been made to list the Diptera according to the time of day and temperature conditions at the time of capture, as is done later for the solitary bees. Flies may be taken for many more hours of the day than solitary bees, because the flies do not return to nests at night or during inclement weather. At cool or cold temperatures they are harder to find and are inactive; so that while they may often be taken at lower temperatures, this does not imply effective work in pollination except at temperatures suitable for flight. It was noted in orchard B that Eristalis abandoned the blossoms when the wind was rather strong and was not often seen at the lower temperatures which occurred during the blooming period.

HYMENOPTERA

For the solitary bees, which are often so important in the pollination of fruit blossoms, certain additional information is of interest. During the progress of collecting, it was noticed that the solitary bees ceased their visits to the blossoms rather early in the day, not being taken abundantly after about 3 p. m. Data for each species of the two more important genera, Halictus and Andrena, showing the distribution by orchards (as for the Diptera), the lowest temperature at which captures were made, and the latest hour of the day when they were caught the average temperature, and time are given in Tables 2 to 5. The tables show that Halictus stops work earlier than does Andrena, perhaps because of the smaller body size of the individual bees of the genus Halictus. It was found as the work proceeded that in late afternoon only the larger bees were to be seen.

TABLE 2.—*Insects of the genus Halictus collected and frequency of each species by orchards*

Species of Halictus	Frequency of each species by orchards indicated							Miscellaneous	Total
	A	B	C	D	E	F	G		
arcuatus			1		1	13			15
brycinci	3	5	3	1	10	3		2	27
cearuleus						1			1
coriaceus		3	3	2	5	6		1	20
divergens				1					1
lerouxii	6	9	4	8	8	25		2	62
lineatulus	3		2	15	31	11	1		63
nubilis				2					2
nymphaearum	1		1		1	2			5
obscurus						15			15
pilosus		1					2		3
provancheri	88	14	20	10	99	34		3	268
quebecensis						1			1
sparsus			2	5		6			13
versatus	1		1	4	12	6			24
(*Chloralictus*) sp	2	1	2	8	8	7		1	29
Total	104	33	39	56	175	130	3	9	549

TABLE 3.—*Temperature and time data in regard to the collecting of the genus Halictus*

Species of Halictus	Temperature		Last hour collected [a]	Hour when most abundant	Average time collected
	Lowest	Average			
	° F.	° F.			
arcuatus	59	70	1.30 p. m.	1.30 p. m.	12.00 m.
brycinci	52	66	1.00 p. m.	12.00 m.	10.30 a. m.
caerulus	71		12.30 p. m.		
coriaceus	55	65	3.30 p. m.	10.00 a. m.	11.30 a. m.
divergens	66		12.00 m.		
lerouxii	55	69	3.30 p. m.	1.00 p. m.	11.30 a. m.
lineatulus	60	67	2.00 p. m.	1.00 p. m.	12.00 m.
nubilis	68		2.00 p. m.		
nymphaearum	59		2.00 p. m.		
obscurus	63	69	10.30 a. m.	8.30 a. m.	9.15 a. m.
pilosus	57		2.00 p. m.		
provancheri	48	73	[b] 4.30 p. m.	10.00 a. m.	11.00 a. m.
quebecensis	83		2.30 p. m.		
sparsus	65	70	2.30 p. m.	2.00 p. m.	12.30 p. m.
versatus	60	66	2.00 p. m.	10.00 a. m.	11.15 a. m.
(*Chloralictus*) sp.	52		3.00 p. m.		

[a] Collections were kept separate hour by hour. The time indicated is the beginning of the hour. The hours of collecting began either on the even hour or the half hour.

[b] This bee was collected at an air temperature of 83° F., with bright sunshine. Omitting this individual, the last hour of collection for this species was 2.30 p. m.

TABLE 4.—*Insects of the genus Andrena collected and frequency of each species by orchards*

Species of Andrena and sex	Frequency of each species by orchards indicated							Miscellaneous	Total
	A	B	C	D	E	F	G		
carlini, females		1	1	11	23	38			74
carlini, males		1	7	3	1	3			15
claytoniae, females					1	4			5
claytoniae, males			1	1					2
crataegi, females						2			2
crataegi, males						15			15
comoda					1	2			3
flavoclypeata, females		1		18	9	32			60
flavoclypeata, males						2		1	3
forbesi, females		1	2	15	28	18			64
forbesi, males	1		6	12	2	2			23
hippotes, females	2	17	9	10	16	18			72
hippotes, males	1	2	2	14	2	4		1	26
mandibularis, females		1		9	4	17			31
mandibularis, male						1			1
milwaukeensis				31					31
multiplicata		1		3				1	5
nivalis, females						5			5
nivalis, males	1	1	3	14		3			22
personata, female				1					1
placida, females	1			3	1	4	2		11
rugosa, females				4	1	1			6
rugosa, male				1					1
vestita, females				1	10	22			33
vestita, males	3		1	3	5	20			32
vicina, females		1		8	18	12	1		40
vicina, males			3	2		2			7
wilkella	6	5	3	1	1	4			20
Andrena spp.			2	3	1	4			10
Total	15	32	40	168	124	235	3	3	620

TABLE 5.—*Temperature and time data in regard to the collecting of the genus Andrena*

Species of Andrena and sex	Temperature		Last hour collected [a]	Hour when most abundant	Average time collected
	Lowest	Average			
	°F.	°F.			
carlini, females	48	64	4.30 p.m.	1.00 p.m.	12.30 p.m.
carlini, males	53	61	2.00 p.m.	10.00 a.m.	11.00 a.m.
claytoniae, females	60		1.30 p.m.		
claytoniae, males	60		12.00 p.m.		
crataegi, females	66		12.30 p.m.		
crataegi, males	59	67	2.00 p.m.	10.00 a.m.	11.00 a.m.
comoda	59		3.00 p.m.		
flavoclypeata, females	59	68	3.00 p.m.	1.00 p.m.	11.45 a.m.
flavoclypeata, males	66		10.30 a.m.		
forbesi, females	49	65	3.00 p.m.	12.00 m.	12.00 m.
forbesi, males	56	64	2.00 p.m.	2.00 p.m.	12.00 m.
hippotes, females	46	66	3.00 p.m.	1.00 p.m.	11.30 a.m.
hippotes, males	54	63	2.00 p.m.	2.00 p.m.	11.20 a.m.
mandibularis, females	57	67	3.00 p.m.	10.00 a.m.	11.45 a.m.
mandibularis, male	67		8.30 p.m.		
milwaukeensis	61	66	4.00 p.m.	2.00 p.m.	1.25 p.m.
multiplicata	62		10.00 a.m.		
nivalis, females	58		4.30 p.m.		
nivalis, males	54	62	2.00 p.m.	9.00 a.m.	11.15 a.m.
personata, female	59		1.00 p.m.		
placida, females	63		2.00 p.m.		
rugosa, females	60		4.30 p.m.		
rugosa, male	59		1.00 p.m.		
vestita, females	48	67	2.30 p.m.	10.30 a.m.	11.00 a.m.
vestita, males	60	69	2.00 p.m.	10.00 a.m.	10.30 a.m.
vicina, females	48	63	2.30 p.m.	1.00 p.m.	12.00 noon.
vicina, males	54		4.30 p.m.		
wilkella	54	75	3.30 p.m.	10.00 a.m.	11.00 a.m.
Andrena spp.	55		2.00 p.m.		

[a] Collections were kept separate hour by hour. The time indicated is the beginning of the hour. The hours of collecting began either on the even hour or the half hour.

An outstanding difference between species of Halictus and Andrena, in comparison with the Diptera, is the fact that the solitary bees build nests and return to them not only to supply the nests with food but also for shelter at night and in bad weather. This doubtless accounts for their absence from the orchards in cold weather and in late afternoon.

A separate tabulation is made for the eight species of bumblebees (119 individuals) taken. (Table 6.) The fact that eight species were taken indicates that the catch was fairly representative. At the time of apple blossoming, only queen bumblebees are to be found, for it is at just this time of year that the queens are seeking locations for their nests or are constructing the very beginnings of the nests. Several queen bumblebees were seen searching for nesting places while this work was under way. Because of the noise which they make when they fly, bumblebees usually receive credit for more effective work in orchards than seems to be due them. Not only are they not abundant at this season, but they often come darting into an orchard, visit two or three flowers, and then depart. Because of their methods of work, they are unusually effective in pollinating those blossoms that they visit. While bumblebees are credited with a fair degree of constancy in their visits to a single species on one trip, their wide range of flight would seem to reduce their value to the fruit grower. The noise which bumblebee queens made as they entered the orchards where collections were being made may have attracted the attention of the collectors and caused a larger number to be taken.

There was in fact some rivalry among the collectors as to which could capture the most bumblebee queens, so that the number is doubtless a little large for these species.

TABLE 6.—*Solitary bees in genera other than Halictus and Andrena, bumblebees, and Hymenoptera other than solitary bees and bumblebees collected and frequency of each species by orchards*

OTHER SOLITARY BEES

Species	Frequency of each species by orchards indicated							Miscellaneous	Total
	A	B	C	D	E	F	G		
Agapostemon (Halictus) radiatus	1	1							2
Agapostemon texanus	1				5	7			13
Augochlora confusa			1	1	16	3			21
Ceratina calcarata		1							1
Ceratina dupla		1							1
Colletes inaequalis						1			1
Nomada obliterata	1								1
Osmia atriventris			2			2			4
Osmia lignaria	1		1	1	1		1		5
Osmia pumila			3			1		1	5
Sphecodes ranunculi	2	2	1	5		1			11
Total	6	5	8	7	22	15	1	1	65
Total for all solitary bees	125	70	87	231	321	379	7	13	1,233

BUMBLEBEES

Bombus affinis							1		1
Bombus bimaculatus		1		2	4				7
Bombus fervidus	2	4	2	3	5	2			18
Bombus impatiens	1	5	5	13	2	14		1	41
Bombus perplexus		2	1	5		12			20
Bombus ternarius						1			1
Bombus terricola		4	3	4	2	5	1		19
Bombus vagans	2		2	4	1	2		1	12
Total	5	16	13	31	14	36	2	2	119

MISCELLANEOUS HYMENOPTERA

Ophion bilineatum								2	2
Ephialtes sp.		1	3						4
Amblyteles atrox (?)			1						1
Amblyteles sp.			1			1			2
Chrysis coerulans (?)				1					1
Polistes pallipes		1							1
Vespa (Dolichovespula) diabolica		1	2			1			4
Vespa (Vespula) maculifrons			1	1	1				3
Xylocopa virginica	3		1						4
Total	3	3	9	2	1	2		2	22

Tabulations are also made of solitary bees other than those of the two genera Halictus and Andrena and for Hymenoptera other than the solitary bees (Table 6), but in these cases records by orchards and totals are given without records of temperatures.

PREVALENCE OF HONEYBEES

While honeybees were not collected, except in a few cases when mistaken for Andrena on high blossoms, some impression as to their prevalence in comparison with other insects was unavoidable. In orchard A, seven colonies of bees, maintained for pollination purposes, were located in or immediately adjacent to the orchard. For some

reason during the two days when collections were being made in this orchard, the honeybees from these colonies were working more in a cherry orchard a little over a quarter of a mile away than they were on the MacIntosh apple trees, which were just coming into full bloom. Later, after the collection of other insects was discontinued, the honeybees worked well on the apple blossoms. In orchard C a few weak colonies of bees had been placed; in orchard D there was one colony of bees; and near orchard F, almost directly across the road, was a small apiary, and honeybees were observed flying freely to and from these hives to the trees of the orchard. In the other orchards no provision had been made to supply honeybees, but a bee tree was discovered not far from orchard B, the colony appearing not to be strong.

The general impression was that in all the orchards honeybees outnumbered all other insects combined at least 3 to 1. In orchard G, noted for the scarcity of wild insects in 1931, the ratio was easily 100 to 1. Honeybees, it may be said, are more valuable in the orchards where these collections were made than all the wild insects together. This appears also to be true for the western New York fruit belt as a whole, based on more casual observations.

The honeybees worked earlier in the morning and later in the afternoon than did the wild bees, but bumblebee queens were often observed or captured at temperatures too low for honeybee flight. Bumblebee queens flew both early and late and were usually about the last insects to be seen each day. Honeybees were considerably more abundant in the middle of each day, at which time bumblebees appeared to be less prevalent.

THE POSSIBILITY OF INCREASING THE WILD SPECIES

It seems probable that the scarcity of wild insects on apple blossoms is due to a combination of factors incident to the agriculture of the fruit districts. The relatively high land values tend to reduce waste land and wood lots and also tend to eliminate the wide fence rows which are favored nesting places for some species. Cultivation reduces nesting and hibernating places, especially of solitary bees. Clean cultivation of orchards, where practiced, still further reduces the opportunities for the propagation of wild bees. It is possible that the efforts of fruit growers to control injurious insects in some degree serves to destroy individuals of those species which are beneficial. Beekeepers have observed that dusting destroys many honeybees, and it is probably equally disastrous to solitary bees.

The question naturally arises whether it would be feasible to make an effort to provide better conditions for wild insects. For the Hymenoptera this does not seem possible in any way that would make a practical appeal to fruit growers. For one species of fly (*Eristalis arbustorum*) already mentioned, there appears to be at least a possibility of increasing the number, as is suggested by the abundance of these insects from the roadside ditch near orchard B. These insects breed in polluted water which is not too deep, and which contains an abundant supply of food for the rat-tailed maggots constituting the larval stage of the species. It might be possible to increase the number of these insects by providing suitable breeding places far enough from residences as not to be offensive. A small preliminary experiment of

this kind was carried out during the summer of 1931, and toward the close of the season many puparia were in the adjacent soil. There are no results of value yet to be reported. Maggots of this species of fly are sometimes present by the millions in slow-flowing small streams or ditches polluted by cannery or milk wastes,[4] especially those with mud bottoms. Within the area of the fruit belt of western New York there are numerous canneries from which such waste might originate, but only one of the orchards (orchard D) from which collections were made was within flight range of such a cannery, and in this orchard Eristalis was not abundant.

CONCLUSIONS

The sparsity of the population of wild insects in seven orchards in Monroe County, N.Y., where collections were made, is striking, but not surprising after some years of casual observations.

On the basis of the observations made, honeybees outnumbered all other insects.

There seems to be nothing that a fruit grower or group of fruit growers may do in a practical way to increase the population of wild insects, with the possible exception of certain syrphid flies.

[4] CLAASSEN, P. W. BIOLOGICAL STUDIES OF POLLUTED AREAS IN THE GENESEE RIVER SYSTEM. *In* N.Y State Conserv. Dept. Sup. to Ann. Rept. (1926) 16, A Biological Survey of the Genesee River System p. 38–46, illus. 1927.

ECONOMIC STATUS OF THE GREEN STINKBUG WITH REFERENCE TO THE SUCCESSION OF ITS WILD HOSTS[1]

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INTRODUCTION

There are frequent records of injury by the green stinkbug, *Acrosternum hilaris* Say, to many species of plants. Green bean pods, bolls of cotton,[2] the fruit of tomato,[3] and the fruit of both peaches [4] and grapes [5] are often attacked. Serious injury is generally local and somewhat periodic. Studies made in Virginia during the past seven seasons indicate that the insect has a distinct preference for certain native hosts and that the abundance of the insect depends on the presence of these plants. Injury to economic crops usually does not occur unless some of the wild plants fail to supply food. In a study of some of the causal factors involved in serious injury to Lima beans, the crop that has suffered most commonly in this state, the following conclusions have been reached: (1) Wild or uncultivated bushes and trees are naturally preferred; (2) a succession of hosts appears to be necessary for the insect to maintain itself in numbers; (3) cultivated crops are apparently damaged only when the wild-host succession is broken; and (4) severe injury to Lima beans is closely associated with a fungous disease organism.[6]

The studies on this pest were made in a farming section about 8 miles north of Richmond, Va. Clumps of trees occur here and there on the farms, and there are numerous tracts of timber. The section is partly bounded by the Chickahominy Swamp, which supports a dense thicket of native trees and bushes. A commercial nursery farm is located in this section. The fields are used for the cultivation of nursery stock and general farm crops, and there are a number of home gardens and home orchards. Practically all the native and cultivated plants of this section are found in this locality, and the bugs therefore had an opportunity to select the food preferred. During the period of time covered by these studies it was possible occasionally to make collections in other parts of the State and to visit farms where serious damage had been reported.

HOST PREFERENCES

In Figure 1 some of the host plants are listed. The adult bugs are occasionally found on mulberry and blackberry in early summer. The other host plants are arranged in the order of apparent preference. The relative rank may vary somewhat for different seasons and

[1] Received for publication Dec. 23, 1932; issued June, 1933.

[2] SANDERSON, E. D. REPORT ON MISCELLANEOUS COTTON INSECTS IN TEXAS. U. S. Dept. Agr., Bur. Ent. Bul. 57:47–49, illus. 1906.

[3] MORRILL, A. W. PLANT-BUGS INJURIOUS TO COTTON BOLLS. U. S. Dept. Agr., Bur. Ent. Bul. 86:78–82, illus. 1910.

[4] WHITMARSH, R. D. THE GREEN SOLDIER BUG (NEZARA HILARIS). Ohio Agr. Expt. Sta. Bul. 310, p. 519–552, illus. 1917.

[5] STONER, D. THE SCUTELLEROIDEA OF IOWA. Iowa Univ. Studies Nat. Hist. 8(4):109. 1920.

[6] WINGARD, S. A. STUDIES ON THE PATHOGENICITY, MORPHOLOGY, AND CYTOLOGY OF NEMATOSPORA PHASEOLI. Bul. Torrey Bot. Club 52:249–290, illus. 1925.

localities. While this insect feeds on a number of plants there are only a few preferred breeding hosts, and not all of these supply food during the main breeding period of adults and throughout the development period of the nymphs. Nearly all adults, eggs, and nymphs collected were on American elder (*Sambucus canadensis* L.), black or common locust (*Robinia pseudoacacia* L.), and honeylocust (*Gleditsia triacanthos* L.), three uncultivated hosts. Only two of these are seasonally well adapted to serve as hosts, namely, elder and honeylocust. Our studies indicate that the insect does not build up a large population when it is dependent on either of these plants. The former has the most regular fruiting habit, but farmers often destroy or cut this plant back to the ground; therefore, for one season at least, food is not available. However, sprouts generally come up and some berries are borne the first season, and an abundance of fruit the second year. Furthermore, where left undisturbed, other more hardy plants and vines tend to crowd out the elder in the course of a few seasons. The honeylocust is well adapted to serve as a host, but in 1929 and 1931

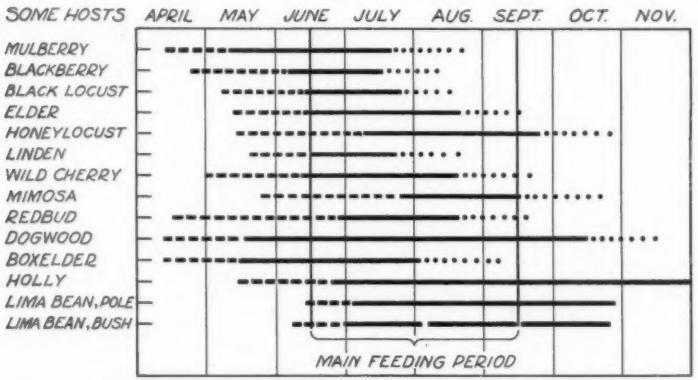

FIGURE 1.—Principal host plants of the green stinkbug and periods during which the seed pods and the fruit are in suitable condition for food as related to the main feeding period of the bug

it had no fruit in this locality. It appears that this tree frequently fails to bear fruit. Wild or black cherry (*Prunus serotina* Ehrh.) and flowering dogwood (*Cornus florida* L.) are natural breeding hosts with fruiting habits that fit in well with the feeding period of the insect, but heavy infestation has not been observed on either of these species. Many American holly trees (*Ilex opaca* Ait.) occur in this locality. The insect feeds on the berries, but it has been collected on holly only at rare intervals.

It may be noted from Figure 1 that several preferred breeding hosts have fruit suitable for food only a part of the season. For instance, black locust is a favorite host, but the pods ripen and dry by early August, about the time the earliest nymphs mature. The same is true of the American linden (*Tilia americana* L.) and American redbud (*Cercis canadensis* L.), both among the preferred hosts. The nymphs perish unless food is supplied the remainder of the period by other plants such as honeylocust, mimosa (*Acacia angustissima* (Mill.) Ktze., var. *hirta* (Nutt.)), dogwood, holly, grape (*Vitis* sp.), peaches (*Prunus persica* (L.) Stakes), or beans located

near enough for the nymphs to reach them by crawling. Hence, not many insects, as a rule, are found where such trees as black locust, redbud, or linden grow alone or together.

Lima beans (*Phaseolus lunatus macrocarpus* L.) are the preferred cultivated host, the pole varieties being more subject to attack than the bush types; however, in the trucking and canning sections where Lima beans are grown extensively, serious injury has not been observed. In most cases where bean fields were examined the insect was not found.

IMPORTANCE OF A SUCCESSION OF HOSTS

It appears that a sequence of wild plants in close proximity is necessary for this insect to maintain itself. Increase in numbers is impossible without such succession. One species of plants, though numerous, is not enough. Two, three, or four species growing relatively close together, or intermixed, appear to be essential. There are several reasons for this: (1) Nymphs are largely dependent on young or immature fruit or pods, and these are much preferred by the adults; (2) the main period during which nymphs are present continues about 12 weeks; and (3) the food supply is often cut short by unfavorable weather or other causes. Ideal conditions are approached where a succession of hosts exists to meet the essential needs of the adults, and to cover the development of all the nymphs. An excellent natural combination or succession appears to be black locust, honeylocust, and mimosa. This combination existed at the nursery in the section studied. Another succession favorable to the insect is found where either black locust, linden, or boxelder (*Acer negundo* L.) occur alone or together near grapes or growing green beans.

ATTACK ON ECONOMIC CROPS

Instances where farm or garden crops have been severely damaged so far as observed in Virginia, occurred where the host succession was incomplete or broken and the cultivated crop happened to be near and in condition to furnish the needed food. Usually the attack was made in August when nymphs migrated to the near-by crop from some wild host which had ceased to furnish food. Two instances of particularly severe attacks on Lima beans in the southern part of the State are given as examples. At Disputanta in 1928 practically all the beans in a home garden were ruined soon after the middle of August. Near by were four large linden trees. Food on lindens became exhausted, and the nymphs migrated to the Lima beans and grapes. In 1929 the Lima beans were planted a few hundred feet distant from the linden trees and were not attacked, although the trees were infested and a migration of nymphs occurred as in the previous season. Grapes and sweet peppers in the garden were severely attacked. There was a distinct migration each year. At Drewryville, Lima beans were ruined in one garden in 1929. Apparently this occurred because elder bushes, which grew in abundance on ditch banks and hedgerows, had been cut during the winter and the honeylocust trees on the farm bore no fruit pods in that season. The elder bushes grew and bore fruit in 1930, and the locust trees were full of pods. In mid-July the insects were very plentiful on these native plants, but none were found on the Lima beans although

they were planted in the same location as in 1929. The locality was visited again in early August, 1931. The bugs were found on elder bushes but none on beans.

Lima beans, in gardens at the nursery north of Richmond, even when planted close to natural host trees were not appreciably damaged, although adults and nymphs were very plentiful on the wild hosts each season. There was observed some tendency for adults to migrate to Lima beans during late July and early August, but this movement was too late for much injury, because the majority of the eggs had been deposited on native tree or bush hosts.

TRANSMISSION OF YEAST-SPOT DISEASE

When Lima beans are attacked, the injury is greatly increased by the presence of the bean yeast-spot organism, *Nematospora phaseoli*. In the two bean fields mentioned above, practically all the pods dropped off by the middle of August. This disease seems to be widespread in eastern Virginia and is generally associated with the feeding of this insect.

NATURAL ENEMIES

Very few natural enemies have been taken in Virginia except egg parasites. The average parasitism of eggs for six seasons at Richmond ranged from 16 to 27 per cent. In some localities as high as 55 per cent were parasitized. Six species of egg parasites have been reared and identified as follows: *Trissolcus euschisti* (Ashm.), *Anastatus reduvii* (How.), *A. pearsalli* (Ashm.), *A. mirabilis* (Walsh), *Telenomus dimmocki* (Ashm.), and *T. podisi* (Ashm.). The first two named were most numerous. Generally *T. euschisti* ranked first, and *A. reduvii* second. *A. pearsalli* was third in numbers but never numerous. The last three species were scarce.

Nymphs and adults are parasitized by tachinids. *Trichopoda pennipes* is the only species reared. Parasitism by tachinids has been very light except in one or two collections when eggs were very numerous on nymphs and adults. In one collection 65 per cent of the adults were parasitized.

SUMMARY

The green stinkbug, *Acrosternum hilaris* Say, which is a single-brood insect, feeds and deposits eggs by preference on wild hosts and requires green pods or fruit for food. The active breeding and development period is about three months which is generally too extended for one host to furnish a continuous supply of food. Studies made in Virginia for seven seasons indicate that a host succession is necessary for the insects to become numerous. Furthermore, it appears that cultivated crops are not severely attacked except when the host succession is incomplete or broken. When the insect feeds on Lima beans, the pods generally become inoculated with a fungous disease which may result in a complete crop failure, especially in humid seasons. Among natural enemies, egg parasites are most important. Six species of egg parasites have been reared and identified.

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